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SUBSEXUAL REPRODUCTION IN *AGROPYRON*

JOHN BRUCE HAIR
Botany Division, D.S.I.R., Christchurch, New Zealand

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1. INTRODUCTION

THE grass *Agropyron scabrum* is found in both islands of New Zealand, in Norfolk Island and in eastern Australia. It is a complex of morphologically distinct populations which preserve their separate character both in nature and in cultivation. They do so because they are self-pollinated and true-breeding, and they form no natural hybrids (Connor, 1954).

A preliminary study of meiosis in this complex showed that whereas in some populations chromosome pairing was normal, in others it was less regular, and in one population there was little or no pairing at all. Four populations were therefore selected for further investigation, the results of which are described in the present paper.

2. MATERIAL AND METHODS

The parental material, derived from four areas (table 1), consisted of 30 selected plants,* which were multiplied clonally in culture and also bred. The seedling progenies totalled 664 plants.

Mitosis was studied in Feulgen squashes of root tips, following pretreatment in α-bromonaphthalene or 0·2 per cent. colchicine (3-4 hours) and fixation overnight in 2BD. For good maceration and staining, hydrolysis at 60° C. was extended to 18-24 minutes.

Meiosis in PMC was examined in temporary acetocarmine squashes, following Thomas's method (1940). Storage was satisfactory in ordinary acetic alcohol at 4-5° C.

Embryo-sacs, embryos and endosperm were traced in paraffin sections of whole buds or excised ovaries after fixing briefly in Carnoy, overnight in CrAF.

The stain was crystal-violet. T.S. and L.S. were cut at an increasing thickness of 16-36 microns.

3. PARENT PLANTS

The main details of morphology and habitat are recorded by Connor (*loc. cit.*).

All populations are normally hexaploid with 42 chromosomes (table 1). The centromeres are all submedian, and as a rule two nucleolar pairs can be recognised with certainty (text-fig. 1*a-b* ; *cf.* plate I, figs. 1-2).

In each parental group (except A, table 1) there were some cytologically abnormal plants whose abnormal character was usually expressed phenotypically. The chromosome aberrations were of two kinds.

(*a*) *Numerical*, including relative triploidy ($2n = 57, 63$; plate I, fig. 1).

(*b*) *Structural*, one chromosome showing a deficiency (text-fig. 1*a-b*).

* From the extensive field collections of Mr H. E. Connor, Botany Division, Department of Scientific and Industrial Research, Christchurch, N.Z.

This preliminary information was of immediate value. It gave some idea of the kind of chromosomal variation occurring in wild populations ; it made possible a provisional sub-grouping into chromosome classes ; and it supplied useful cytological markers. Finally,

TABLE

Versatile reproduction in gro

Wild population parents : 30 plants							
Group and family	No. of plants	2n	Male		Female		Endosperm
			Meiosis	n	Type of division	Presumed n	
North Island A	5	42 (6x)	21 II	21	As male		3n
B1	4	42	I-IV	20-22	As male		±3n
2	1	43	I-IV	21-23	As male		...
3	1	63	I-V	*ca.* 31	Meiosis Dyads ?, mitosis ?	63	...
South Island C1	2	42	18-21 II Rare IV	±21	Meiosis Dyads, mitosis	? 42	... ...
2	1	57	I-III	*ca.* 28	Dyads, mitosis	57	...
3	1	63	I-III Rare V	*ca.* 31	Dyads, mitosis	63	...
D1	2 normal	42					...
2	2 deficient	42	Intraplant	42 or ±21	Dyads, mitosis	42	±6n
3	10 normal	42	0-8 II				...
4	1 deficient	42					...

A : Wellington, N.Z. B : Foxton, N.Z.
C : Dunstan, N.Z. D : Waiau, N.Z.

it led to the suspicion that different modes of reproduction were operating in the different populations.

This expectation was then tested in two simple ways : by raising large progenies and comparing them with their respective mother-plants ; and by pollination experiments.

4. PROGENIES

The results of progeny testing are summarised in table 1. Since there were no differences between sister-families raised by isolation and by open-pollination, the two sets of data were pooled.

ABLE
ion Agropyron scabrum

Progenies (isolation and open pollination) : 664 plants					Genetic mode of reproduction of parent
No. of plants	With parental chromosome no.	Aberrants			
		Chromosome no.	Presumed origin	Per cent.	
25	100 per cent.	...	...	...	Sexual
74	75·7 per cent.	n ? n, $n+1$ $2n-1$, $2n+1$ or 2 $3n$, $3n+3$	(Sub-lethal) Haploid parthenogenesis Sexual Semi-sexual	6·8 4·0 8·1 5·4	Facultative apomixis
46	34·8 per cent.	n $2n$, $2n+2$ to 3	Haploid parthenogenesis Sexual	4·4 60·8	
5	0	$4{\cdot}5n$ (*ca.* 96)	Semi-sexual	100	
201	99 per cent.	$2n-1$ *	Diploid parthenogenesis	1·0	Predominant apomixis (sub-sexual)
1	(100)	...	...	...	
13	92·3 per cent.	$4{\cdot}5n$ (94)	Semi-sexual	(7·7)	
95	96·8 per cent.	$2n-1$,* $2n+1$ †	Diploid parthenogenesis	5·0	Obligate apomixis
60	88·3 ,,	$2n$,‡ $2n+1$, $2n+2$, $4n$			
118	96·6 ,,	$2n-1$, $4n$			
26	96·1 ,,	$2n+5$ §			

* one chromosome dicentric
† one short chromosome
‡ two short chromosomes
§ centric ring

A : Wellington (25 *plants*)

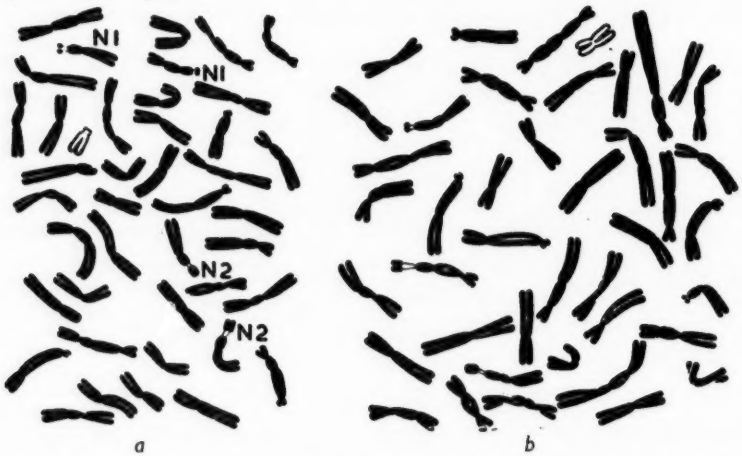
All five plants were self-fertile and gave uniform progenies without aberrations in chromosome form, number or behaviour. Further, they were successfully crossed with other species and varieties giving undoubted hybrids (table 2). Clearly this group is entirely *sexual*.

B : Foxton (125 *plants*)

The six parental plants were sister-plants, one generation removed from the wild type. Three other plants of the same family—two male-sterile and one " haploid " and sterile—set no seed. The next generation (ex B1 and 2) showed the same variability. " Haploids " ($3x$) and " triploids " ($9x$) were regularly produced, as well as a high proportion of unbalanced forms. Both meiosis and fertilisation were liable to fail but in an uncoordinated way with great loss of fertility.

The third chromosome class (B3) gave exclusively higher polyploids with about 96 chromosomes, presumably by fertilisation of unreduced egg-cells. In this lineage, failure of reduction was repetitive.

The group as a whole is evidently subject to non-recurrent disturbances in sexual reproduction ; in other words, it is semi-sexual or *facultatively apomictic.*



TEXT-FIG. 1.—Somatic complements ($2n = 42$) of the obligate apomicts, D2 and D4, each showing one short, deficient chromosome. ×2000.
N1 and N2 : the two pairs of nucleolar chromosomes.
(*a*) D2 ; (*b*) D4.

C : Dunstan (215 *plants*)

Here the identity of each parental sub-group, even when aneuploid, was preserved in the progenies. The only significant exception was in the progeny of C3 : one plant among 13 arose *semi-sexually, i.e.* by failure of meiosis but not of fertilisation. The single aberrant which appeared in the progeny of the hexaploid (C1) arose *sub-sexually, i.e.* parthenogenetically, following a suppressed meiosis in which crossing-over and segregation occurred despite the failure of reduction. It contained an isodicentric nucleolar chromosome, the remarkable properties of which have been described elsewhere (Hair, 1953).

Taken together the results indicate that the different Dunstan units are *predominantly apomictic.*

D : Waiau (299 plants)

Taking the whole progeny of the 15 parental plants we find 284 entirely like the parents and 15 which had aberrant numbers and even forms of chromosomes. Again, one had a dicentric and one had a ring, common results of inversion crossing-over. Thus the mode of reproduction was not giving stability, indeed less stability than would sexual reproduction in a polyploid. Yet the two parents with single deficient chromosomes (D_2 and D_4) had together 86 seedlings, every one of them carrying the deficient chromosome of the parent in the same heterozygous condition as the parent except one which had it in the homozygous condition, and one " tetraploid " ($12x$) which had it in double dose.

This result likewise contradicts our experience of sexual inheritance in polyploids. Both the maintenance of the parental deficiency and the introduction of new aberrations are, on the other hand, consistent with a suppression of reduction combined with a failure to suppress the irregularities of crossing-over and segregation that go with a suppressed meiosis. Obligate apomixis have been attained but subsexual recombination and irregularity remain.

The next task was to test these findings by pollination experiments.

5. POLLINATION EXPERIMENTS

(i) *Method*

Tests for apomixis were mainly confined to the supposed obligate apomicts (D), which had the advantage of being cytologically marked. Genetic markers were not available.

Plants were self-pollinated (particularly the deficiency heterozygotes), and also pollinated by various sexual species, both endemic and foreign, differing in chromosome number from the seed parents under test. Double controls were employed, as follows :

(*a*) Emasculated, isolated flowers.
(*b*) Controlled crosses between sexual species, comparable to the test crosses above.

(ii) *Results*

The emasculated controls of the predominant and obligate apomicts, a total of 1600 flowers, failed to set seed. The control crosses of sexual forms (table 2) in every case yielded the appropriate hybrids and this confirmed the normal sexual character of A populations.

Both self- and cross-pollinations of the obligate apomicts (D) gave almost entirely uniform progenies of strictly maternal character. There were only four aberrants in 106 plants, and these, as will be shown later, were probably due to maternal, subsexual irregularities in embryo-sac development.

Although the kind of pollen used had no effect upon the *character* of the progenies, it had a marked influence on the *numbers* of plants

produced. Self-pollen was more effective than cross-pollen. In cross-pollinations, the most effective male was the octoploid, next the hexaploid, and last the tetraploid. The reason for this gradation will be considered in section 9.

No seed was formed when Waiau families were pollinated by the foreign tetraploids *A. cristatum*, *A. desertorum* and *A. spicatum*, or by the decaploid *A. elongatum*. But the last two succeeded to a limited degree

TABLE 2

Results of self- and cross-pollination of sexual and apomictic forms of A. scabrum, *being the progenies of parents or seedlings in table 1 (408 seedlings)*

♀ parent	♂ parent	Flowers pollinated	Seed (total)	Progeny			Chrom. ratio : endosp./ embryo
				Maternal	Non-maternal		
					No.	Constn.	
A : Sexual :							
6x . .	Selfs	432	207	200	...	...	1·5
	4x	129	12	0	7	F_1 (5x)	1·3
	6x	270	77	0	74	F_1 (6x)	1·5
	8x	162	22	0	21	F_1 (7x)	1·7
D : Apomictic :							
6x+1 . .	Self	24	10	8	1	6x+2 def.	3
6x−1 . .	Self	20	6	6	0	...	3
6x def. .	Self	20	6	6	0	...	3
6x . .	4x	270	6	2	0	...	2·3
6x double def.	4x	44	6	1	0	...	2·3
6x . .	6x	148	11	7	0	...	2·5
6x def. .	6x	149	3	3	...	...	2·5
6x+1 def. .	6x	54	2	...	...	...	2·5
6x . .	8x	520	66	55	2	6x+1 6x−1 *	2·7
6x def. .	8x	173	16	13	1	12x	2·7
6x+1 def. .	8x	79	1	1	0	...	2·7

* One chromosome dicentric.

Def. = Deficient chromosome.

Male parents in cross-pollinations as follows :

4x : *A. enysii.* 6x : *A. scabrum ; A. kirkii.* 8x : *A. tenue.*

in stimulating endosperm development (table 6) and in more extensive trials might have resulted in mature seed.

These results permit two general conclusions :

(*a*) That obligate apomixis undoubtedly occurs in these populations.

(*b*) That seed production depends upon suitable pollination, that is to say, our populations of predominant and obligate apomicts are *pseudogamous*.

These were presumably the end-results of a gradual series of evolutionary changes, each a step in the breakdown of sexual reproduction. What these steps were is already implicit in the behaviour of the different populations described. When and how they occurred are the questions we must now endeavour to answer. For this purpose, we must examine the development of the embryo-sac, embryo and endosperm. Finally, we must determine the influence, if any, of the male gamete in the life cycle.

6. DEVELOPMENT OF THE EMBRYO-SAC (E.S.)

(i) *General*

The ovary has a single anatropous ovule, which consists of an elongated nucellus, reduced to one layer at the apex, and two double-layered integuments. The embryo-sac mother-cell (E.M.C.) is derived directly from a single hypodermal cell of the nucellus.

The E.M.C. are rarely " caught " in the act of division, and in most ovules the occurrence of meiosis (or its breakdown) must be inferred from later (or earlier) events. The most reliable criterion of a preceding meiosis (*cf.* Kiellander, 1937 ; Esau, 1946) is the presence of intact or degenerating megaspore tetrads. Their absence, on the other hand, indicates *ameiosis*. On this basis, the four reproductive groups already delimited in *Agropyron* fall conveniently into two main classes, one sexual or mainly so, the other apomictic.

(ii) *Sexual development (A and B : Table 3a)*

A plants have a normal meiosis (plate II, fig. 1) and most ovules can be classed on sight as sexual (table 3).

B1 (*6x*). Meiosis is complicated by a mixture of uni-, bi-, and multi-valent associations. Also since " triploids " (*9x*) occur in the progenies (table 1), meiosis must occasionally be suppressed.

B3 (*9x*). The original triploid plant was lost but development was examined in three triploid derivatives ex family B1. In three out of ten ovules the resting mother-cell was highly vacuolate and closely resembled the " mitotic " resting cells to be described later in the apomicts. In the remaining seven there was no evidence for a suppression of meiosis, although all progeny of the original triploid (table 1) were probably due to non-reduction. The triploids in general appear to have a capacity for apomictic reproduction which is already implicit in their own derivation from relatively diploid plants. Their apomixis, however, is facultative and constitutes a link between the sexual and predominantly apomictic populations.

The result of meiosis is a T-tetrad. The chalazal megaspore forms the E.S. which is thus monosporic (text-fig. 2*a*). It undergoes three mitoses to form an 8-nucleate E.S. Meanwhile the three sterile megaspores degenerate but their remains are visible adjacent to the E.S. up to its 4-nucleate stage. The growing sac begins to pierce

the enveloping layers of the nucellus (except at the apex) at the 2-nucleate stage.

The primary antipodals divide several times to the number of 12, 24 or even more, cells. At first regular and meristematic, the individual cells rapidly grow into irregular, vacuolate and swollen units with densely staining contents, and they ultimately form a massive tissue occupying the greater part of the chalazal region and encroaching more or less on the micropylar half of the sac. The location of this tissue in the direct path of incoming nutrients, its glandular appearance, and its later behaviour in relation to pollination and endosperm development, leave no doubt that its function is nutritive (*cf.* Brink and Cooper, 1944 ; Beaudry, 1951).

Organisation of the egg apparatus and polar nuclei follows a normal course. The polar nuclei lie in contact but do not form a true fusion-nucleus before pollination.

(iii) *Apomictic development (C and D : Table 3b)*

In the C plants only four of 191 ovules showed evidence of meiosis (table 3*b*), and these were confined to a single aberrant clone (ex C1). This rare meiosis was probably abortive : no megaspore tetrads or remains of them were seen throughout the clone or elsewhere in the three C populations jointly considered.

Ameiosis, on the other hand, affected at least 96 per cent. of the total material. Two distinct processes, the second being the more frequent, were involved : (1) Dyad Formation following a suppressed meiosis and restitution, or (2) Simple Mitosis without any trace of a meiotic prophase (*cf.* table 3*b*).

Dyad Formation. In other apomictic plants this has been attributed to two kinds of antecedent processes. The mother nucleus, which is more or less asynaptic, undergoes either :

(*a*) An abortive " pseudo-heterotypic " division, which lapses at anaphase into a single restitution nucleus, and is followed by an effective equational division (Osawa, 1913 ; Okabe, 1932 ; Bergman, 1941 ; Rosenberg, 1927) ; or

(*b*) A " pseudo-homeotypic " division in which the unpaired chromosomes, contracted as in an ordinary meiosis, pass straight into division (Gustafsson, 1934).

In *Agropyron*, the first sequence is probable, although only its later stages have been directly observed (table 3*b*). The main criterion of restitution was taken to be the constricted form of the nucleus (text-fig. 2*b* ; plate II, fig. 2).

In all observed dyads save one, the chalazal cell enlarged to form the E.S. initial, and the smaller micropylar cell degenerated (text-fig. 2*d-e*). In the odd dyad, the order was reversed (text-fig. 4*b*). This is enough to indicate between the two cells the Renner Effect. Where a 2-

TABLE 3

Summary of observations showing the sexual, miiotic, and restitutional methods of origin of the embryo-sac in different populations

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Summary of observations showing the sexual, mitotic, and restitutional methods of origin of the embryo-sac in different populations

(*a*) Sexuals and facultative apomicts

Group and family	2*n*	Total ovules	E.M.C.					E.S. with megaspore remnants		Estimated per cent.	
			Resting	Pro.	M I	M II	Tetrads	1-4 nuclei	8 nuclei	Meiosis	Mitosis
A : Sexual . . .	42	52	4	4	1	1	31	9	2	100	0
B : Facultative apomicts :											
B1 . . .	42	12	3	1	2	...	3	3	...	100	0
B3 (1) . . .	63-66	10	3 (2)	2	1	...	4	...	...	70	30 ?

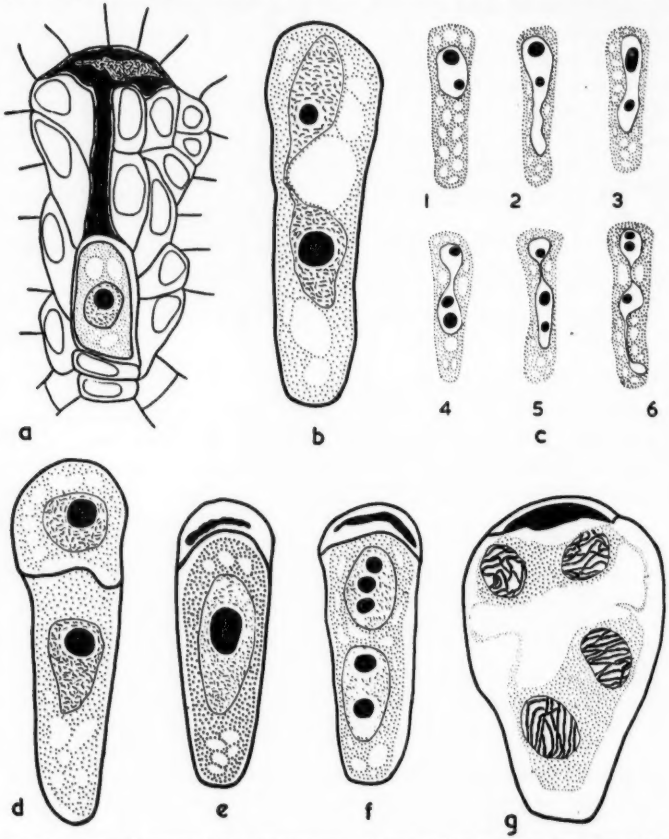
(*b*) Predominant and obligate apomicts

Group and family	2*n*	Total ovules	Mitotic E.S.			Restitution E.S.			E.M.C. (sexual)	Estimated per cent.		
			Resting vacuolate	Pro.-Ana. (3)	2-4 nuclei	Restitution	Dyads	2-4 nuclei	Diak.-AII	Meiosis	Dyads	Mitosis
Predominant apomicts :												
C1 . . .	41-42	85 (4)	15	9	12	1 ?	30	5	4	4·7	42·4	42·4
C2 . . .	57	20	3	1	14	2	...	...	...	0	10·0	90·0
C3 . . .	63	66	20	6	18	8+1 ?	12	1	...	0	33·3	66·7
C4 * . . .	94	20	15	1	4	...	...	...	...	0	0	100
Obligate apomicts :												
D1, 2 . . .	42-43	149	25	20	20	...	68	16	...	0	56·4	43·6
D4 . . .	42	11	1	5	2	...	3	...	...	0	27·3	72·7

(1) 3 plants. (2) Vacuolate. (3) 2 cells at anaphase. (4) 9 cells not classifiable.

* Presumably an obligate apomict arising in the predominant apomict population.

or 4-nucleate E.S. was associated with remnants of the abortive micropylar cell, it was classified as "restitutional" (table 3*b*), *i.e.* as originating from a previous dyad stage (text-fig. 2 *f-g*; plate II, fig. 4).



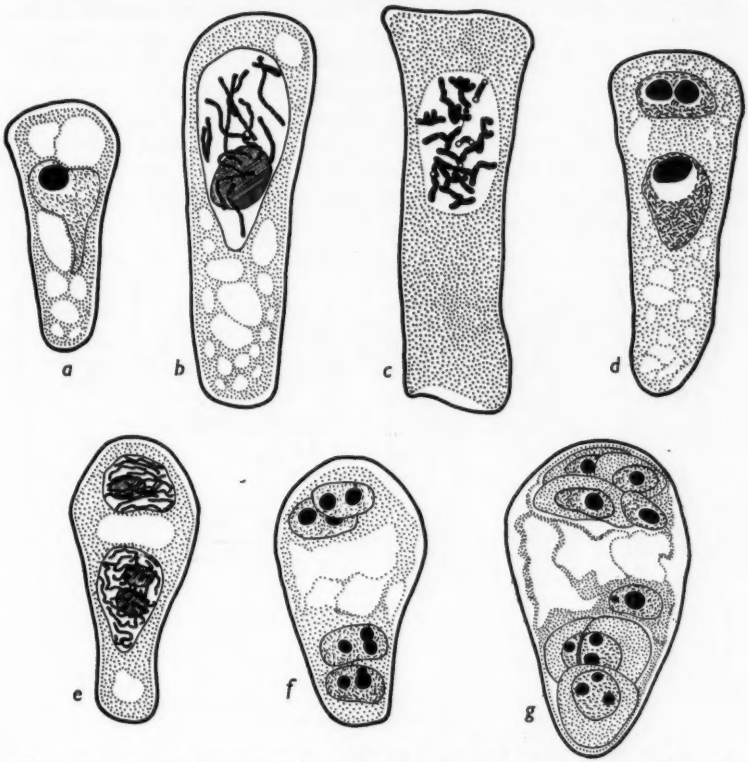
TEXT-FIG. 2.—Development of the embryo-sac. Upper end micropylar, lower one chalazal. ×750 : (*c*. ×350).

(*a*) Normal sexual development. Chalazal megaspore the definitive E.S.
(*b*) Restitution nucleus.
(*c*) 1-2 Resting nucleus in mitotic E.S. 3-6 Restitution nuclei.
(*d*) to (*g*) Successive stages following dyad formation.
(*e*) 1-nucleate.
(*f*) 2-nucleate.
(*g*) 4-nucleate E.S. with remains of abortive micropylar cell still showing.
(*a*) Facultative apomict B1 ($2n = 42$).
(*b*) Predominant apomict C2 ($2n = 57$); (*c*) C3 ($2n = 63$); (*d*) to (*e*) C1 ($2n = 41$-42).
(*f*) to (*g*) Obligate apomict D2 ($2n = 43$).

The incidence of dyads varied within and between the two populations. It was 100 per cent. in plant D2, averaged 33·3 per cent. in four triploids C3, but was nil in the high polyploid C4 of the same family.

Simple Mitosis. The evidence for a purely mitotic origin of the E.S. appears at successive stages of division in the mother-cell (table 3*b*).

In the first stage there is the *resting* E.M.C. that is to undergo mitosis. It is large, elongated and highly vacuolated, broad at the micropylar end (text-fig. 3*a* ; plate II, fig. 5). Its large nucleus tended to follow the shape of the cell. These characteristics were accentuated



TEXT-FIG. 3*a-g*.—Development of the embryo-sac by mitosis in the predominant and obligate apomicts (C1, D1, 2 and 3). Upper end of sac micropylar, lower chalazal, in all figures. ×800 to ×1100. See text.

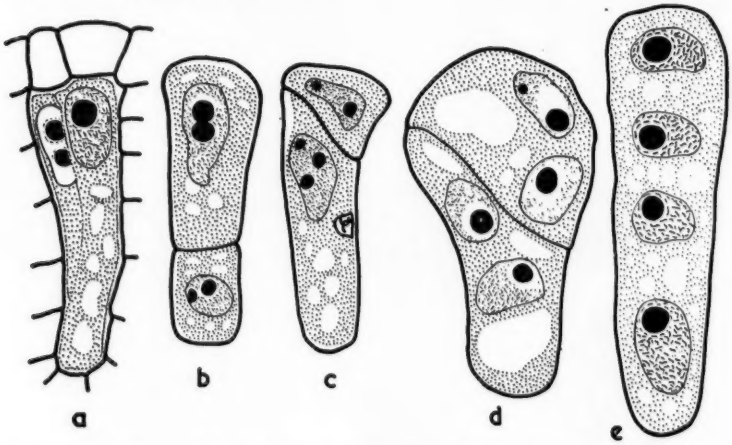
with age, and in most fixations they predominated (41·4 per cent.). The mother-cell evidently passed through a prolonged stage of growth and vacuolation before mitosis began. It finally divided by ordinary mitosis when it had already acquired the character of a uninucleate E.S. (*cf.* Stebbins, 1932 ; Keillander, *l.c.* ; Hakansson, 1943 ; Esau, *l.c.*).*

* The distinction between mitotic and restitutional nuclei was not certain at this stage. The two queried examples of restitution (table 3*b*) were of the same kind ; apart from their shape, both strongly resembled late somatic prophases (*cf.* figs. 2 and 3, plate II). Of the six resting nuclei shown in fig 2*c*, numbers 1-2 were classed as mitotic, numbers 3-6 as restitutional, but the extreme range in shape in this series indicates the possibility of misinterpretation. The difference between a supposed mitosis and the division following restitution is clear only in perfect median sections of the ovule.

The second stage is late prophase, metaphase or anaphase (text-fig. 3*b-c* ; plate II, fig. 3). The chromosomes are then only slightly more contracted than in mitosis of the nucellus. The third stage is the typical 2- or 4-nucleate-sac. It shows no trace of an earlier dyad structure (text-fig. 3*d-f*).

Summarising C and D : normal meiosis on the female side rarely occurs and never effectively. Effective development may be from dyads or by simple mitosis. One of the two dyad cells, which compete, gives the E.S. With mitosis all the products of division in the E.M.C. contribute to the E.S. : there is no formation of spores.

However established, the E.S. followed the same developmental sequence as in sexual plants, but with one important difference. Before, or by the time of anthesis, the unreduced egg had often developed precociously into a small proembryo : it had already put itself out of reach of fertilisation.



TEXT-FIG. 4.—Abnormalities in the early development of the embryo-sac in predominant and obligate apomicts.

(*a*) Two E.M.C. side by side (C2).
(*b*) Micropylar cell of dyad functional (D2).
(*c*) Dyad with oblique cell wall and one chromosome of chalazal cell in a microcyte (D1).
(*d*) Both cells of dyad developing (D1).
(*e*) 4-nucleate E.S. with linear arrangement of nuclei (C3). ×900.

(iv) *Early abnormalities*

In the C and D populations a number of abnormalities are observed prior to anthesis (table 4 and text-fig. 4). Other abnormalities originate during this period but are not seen or fully expressed until later.

7. EMBRYO AND ENDOSPERM

(i) *Sexuals and facultative apomicts (A and B)*

At anthesis the E.S. showed a well-defined resting egg and was in all respects normal (table 5). The free-nucleate endosperm was

usually initiated within 18-24 hours after pollination and in advance of divisions in the young zygote (table 6). Thus all E.S's. scored up to 48 hours after pollination had from 2-16 endosperm nuclei

TABLE 4

Abnormalities in the early embryo-sac development of predominant and obligate apomicts

Group	Example	Abnormality	Times observed	Fig. 4
D1 and 2	1	Micropylar cell of dyad, the definitive E.S.	1	*b*
" "	2	Oblique cell wall in dyad	2	*c*
" "	3 *	Both cells of dyad developing; wall oblique	4	*d*
" "	4 †	Microcyte in chalazal cell of dyad (origin $2n-1$, etc., aberrants)	1	*c*
C1, 2	5 *	Two mother-cells side by side	3	*a*
C3	6	Linear order of nuclei in 4-nucleate E.S.	1	*e*

* Showing effects of competition between normal and supernumerary cells, the latter being usually retarded.

† Most simply explained as the result of partial restitution, giving rare hypoploid aberrants.

but only one-sixth of them showed activity in the fertilised egg. This lag was maintained at 72 hours with the endosperm at least 32-nucleate and the embryo 4-celled; and at six days with the endosperm mainly cellular and the embryo 16-celled.

TABLE 5

Variation in stages of development of the embryo-sac at the time of anthesis

Group	No. of E.S.	No. with Egg+P.N.	No. with proembryo+P.N.			Per cent. precocious
			Pro.-Telo.	2-4 cell	4-8 cell	
A: Sexual .	10	10	0	0	0	0
B: Fac. apo. .	8	8	0	0	0	0
C: Pred. apo.	20	13	1	4	2	35·0
D: Oblig. apo.	23	12	0	11	0	47·8

P.N. = polar nuclei.

The *endosperm*, mainly peripheral up to 72 hours after pollination, then began to concentrate near the proembryo and chalaza, and to form cell walls. After six days, there was little free-nucleate activity and the mass of endosperm became packed with starch.

The *antipodals*, at first greatly stimulated by pollination, declined as the endosperm expanded, the cells being compressed between the endosperm and nucellar tissue, and finally broken up and absorbed.

(ii) *Apomicts (C and D)*

At anthesis up to 50 per cent. of the embryo-sacs had an autonomous and precocious embryo of 1-8 cells (table 5, plate II, fig. 6). This confirmed apomixis and provided a quick means of assay in wild plants whose history was unknown. The same effect was shown by emasculated flowers in isolation: in both predominant and obligate apomicts, the embryo developed for as long as six days after emasculation. At this stage, one plant (C1) had a 32-cell embryo, two polar nuclei and a normal antipodal tissue.

On the other hand, no endosperm developed until 18-24 hours after pollination, and in the absence of pollen it did not develop at all. Pollination, as we saw earlier, is needed for seed production. The reason is now obvious; it is needed for the production of endosperm, in the continued absence of which early autonomous growth of the embryo ceased and the young seed starved and died. We shall see later how the pollen worked.

In apomictic plants there was little correlation between rate of development of the embryo and that of the endosperm (table 6) even long after pollination. These observations confirm the experience in *Parthenium*, and in other apomictic plants (Esau, 1946).

Cross-pollination by endemic, as well as foreign, species of *Agropyron*, though less effective than selfing or open-pollination in stimulating development, results in the same inconsistent pattern of behaviour.

(iii) *Abnormal embryo-sacs after pollination (B, C and D)*

Functional embryo-sacs differ from those of sexual plants only in the precocious development of the embryo. But a proportion of non-functional sacs among apomicts reveal a range of abnormalities (tables 6 and 7).

(a) *Failure of pollination*, or, as we shall see, failure of fertilisation of the polar nuclei, is probably a common cause of non-development.

(b) *Degeneration*: the contents of the E.S. sometimes partly or wholly degenerate; sterile sacs are found with endosperm but without egg or embryo.

(c) *Abnormal stimulation of the antipodals* was expressed in two ways: small supernumerary meristematic antipodals were sometimes found at the boundary of the normal large glandular cells, suggesting a recent burst of mitosis. Again, one, two or even more, isolated cells were actively dividing. This was more pronounced in the cross-pollination *D2* × *A. elongatum* (*10x*) than elsewhere; several cells were affected and some were clearly endopolyploid.

(d) *Correlated irregularities* in the egg apparatus, polar nuclei and antipodals are by far the most frequent. They are recorded in a

TABLE 6

Relative development of embryo and endosperm in the four populations, fixed 18 hours to 9 days after pollination

Group and family	Total E.S.	Egg		No egg +Endosp.	Proembryo	
		+P.N.	+Endosp.		+P.N.	+Endosp.
A : Sexual .	7	0	*3*	*0*	0	*4*
B : Fac. apo. .	20	0	*11*	*4*	0	*5*
C : Pred. apo.	56	44	*2*	*2*	6	*2**
D : Oblig. apo.	181	20	*23*	*22*	49	*67†*

Note : Fourteen E.S. were from cross-pollination of C1 by 4*x* and 61 from cross-pollination of D2 by 10*x*, 4*x* and 6*x*.

Pollen parents in cross-pollinations were as follows :—

4*x* : *A. enysii, spicatum* ; 8*x* : *tenue* ;
6*x* : *kirkii, scabrum* ; 10*x* : *elongatum*.

The relative developments of embryo and endosperm were the same after crossing and selfing.

* Including one of the 14 E.S. from the "cross" C1 by 4x.

† Including 13 of the 61 E.S. from the "crosses" D2 by 4x, 6x and 10x.

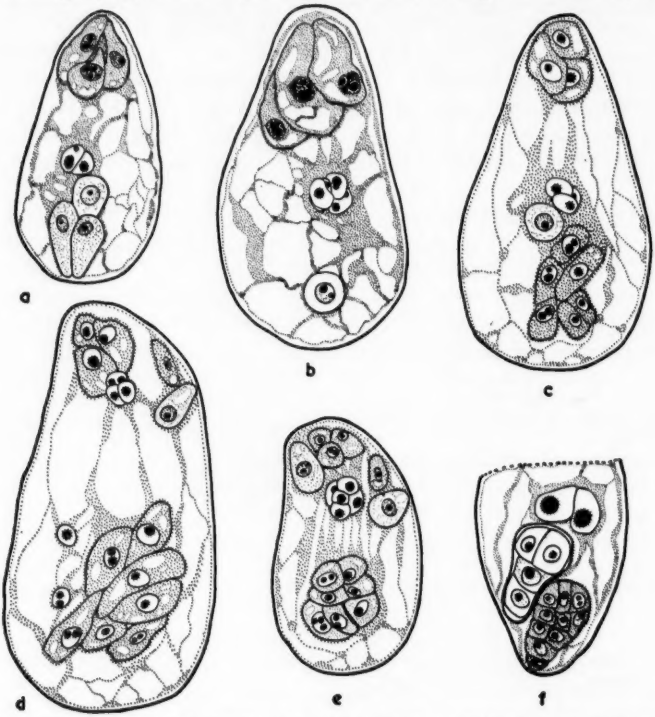
TABLE 7

Classification of mature embryo-sacs, showing the frequency of correlated abnormalities

Group and family	No. of E.S.	No. abnormal	Type or locus of abnormality				Per cent. abnormal
			Polarity	Antip.	Egg app.	P.N.	
A : Sexual .	17	0	...	...	...	...	*0*
B : Fac. apo. :							
2 . .	23	0	...	...	...	...	*0*
3 . .	8	3	3	3	2	3	*37·5*
C : Pred. apo. :							
1 . .	48	3	1	1	1	2	*6·3*
3 . .	11	1	1	...	1	...	*9·1*
4 . .	9	3	0	0	2	3	*33·3*
2 . .	31	16	11	11	8	9	*51·6*
D : Oblig. apo.:							
3 and 4 .	41	1	1	1	...	...	*2·4*
1 . .	65	7	3	3	5	5	*10·8*
2 . .	122	16	14	14	7	7	*13·1*

range of embryo-sacs fixed immediately before anthesis and at intervals up to 72 hours later. They comprise 15 per cent. of 340 E.S's.

As a rule, the different chromosome classes within each apomictic group showed the same range and types of abnormalities, and most had a high proportion of perfectly normal, though unreduced, sacs.



TEXT-FIG. 5.—Abnormal differentiation of the embryo-sacs in facultative and obligate apomicts.

(*a*) Reversed polarity with egg-like synergids. 24 hours after anthesis. D2. *Cf.* fig. 6 (3).

(*b*) Reversed polarity. No synergids ; 4 polar nuclei. 30 hours after anthesis. D2. *Cf.* fig. 6 (13).

(*c*) Central egg, two groups of antipodals at opposite poles and 3 polar nuclei. No trace of synergids. 29 hours after anthesis. D1. *Cf.* fig. 6 (12).

(*d*) Displaced egg and one synergid above ; 3 polar nuclei ; two groups of antipodals. Note the two polar-like nuclei near chalazal antipodals. 20 hours after pollination by *Agropyron elongatum* ($2n = 70$). D2. *Cf.* fig. 6 (11).

(*e*) Two eggs, one displaced, and one synergid ; 5 polar nuclei ; two groups of antipodals. At anthesis. B3.

(*f*) Two embryos side by side in the same embryo-sac. 24 hours after anthesis. D2. ×250 (rest ×150).

But three classes were exceptional : B3 ($2n = 63$), C2 ($2n = 57$), and the high polyploid C4 ($2n = 94$). C3 ($2n = 63$), on the other hand, had no more abnormalities than most of the hexaploid families. Hence an increase in chromosome number does not in itself affect the regularity of the embryo-sac. Of greater significance is the fact

that these classes stand midway between the stable extremes of normal sexuality and absolute apomixis. Their instability in all phases of development is therefore to be expected.

In most cases there is a decrease in the number of synergids, and an increase in the number of polar nuclei, eggs or antipodal groups.

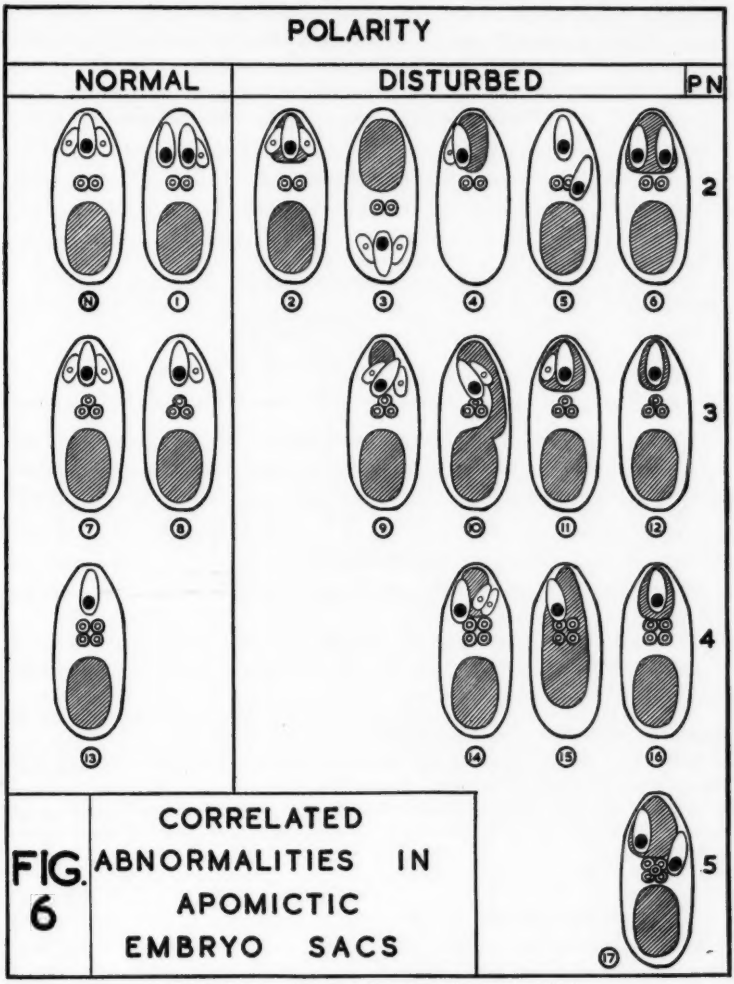


FIG. 6 CORRELATED ABNORMALITIES IN APOMICTIC EMBRYO SACS

Note: Egg nucleus solid, antipodals cross-hatched.

The variations are in position and differentiation rather than in number of nuclei (table 8). They are, moreover, the kinds of variation used in the general classification of types of angiosperm embryo-sac used by Darlington and Mather (1949, fig. 50). Since the primary nuclei

of the 8-nucleate sac are genetically identical, these variations will have no genetic effect.

Similar changes have been reported by Chiarugi and Francini (1930) in apomictic *Ochna serrulata*, and by other workers in different plants (*cf.* Maheshwari, 1950).

TABLE 8

Negative correlation of varying components of the embryo-sac

P.N.	Normal polarity Synergids			Disturbed polarity Synergids		
	2	1	0	2	1	0
(i) Predominant apomicts						
2	N	1	0	1	2	0
3	2	4	0	0	1	1
4	0	0	2	0	0	1
(ii) Obligatory apomicts						
2	N	1	0	7	0	1
3	0	3	0	1	2	1
4	0	0	2	0	0	2

Note.—Analysis of 25 per cent. of the sacs was prevented for the following reasons :—

(i) Rapid division of the primary antipodals hinders or prevents the detection of supernumerary divisions such as may occur in other components at the same time (examples 14 and 17, fig. 6).

(ii) Varied disposition of the antipodals themselves increases the difficulty by tending to obscure the normal micropylar contents of the sac.

(iii) The synergids degenerate at different times, and even in normal sacs their recognition is not always certain. Hence their absence at a particular moment, even when associated with a corresponding increase in the number of polar nuclei, eggs, or antipodal groups, cannot be taken as conclusive proof of their earlier transformation.

(e) *Disturbed polarity.* In 36 of the 50 abnormal E.S's., the polarity was disturbed in one or other of the four main ways shown in table 9. The antipodals were prominent in most of these disturbances. Thus they invaded the micropylar end and tended to displace the egg apparatus (table 9, class 2) ; or they were crowded into the micropylar end of undifferentiated sacs (class 3). When two antipodal groups were present (class 4), the ancillary, micropylar group was usually smaller than the normal, chalazal group. This anomaly was the

most frequent, and hitherto it has been reported only in *Poa alpina*,* as an exceptional event.

The abnormal ovaries of all kinds, in relation to the development of the embryo-sac (although not necessarily in relation to anthesis), were undersized. With one possible exception, the embryo-sacs were probably sterile. The exception, fixed at 20 hours after anthesis, had a small proembryo and endosperm ; here, abnormal development was confined to differential growth of the antipodals. Sterile sacs

TABLE 9

The kinds and frequencies of disturbed polarity in 50 abnormal embryo-sacs

(Three types of apomicts combined)

Class	Situation of the antipodals	Proportion abnormal
1	Polarity reversed . . .	6 per cent.
2	Differential growth . . .	16 „
3	Unipolar (micropylar) . .	16 „
4	Two distinct groups . .	34 „

had no endosperm, but from 2-5 polar nuclei. It is unlikely that a surfeit of polar nuclei, by itself, prevented either fusion or fertilisation. Rather it was yet another symptom of a general upset in development.

(iv) *Twin embryo*

In an obligate apomict two embryos were found side by side in one E.S. (text-fig. 5*f*). Though the possibility that one embryo arose adventitiously from the nucellus cannot be ignored, it seems more probable that it developed from an extra egg cell, the two embryos being in effect identical twins : in this case monosporic although not monozygotic. Several abnormal embryo-sacs, as we saw (text-figs. 5, 6) contained an extra egg which we supposed was a transformed synergid.

8. MEIOSIS IN P.M.C.

A : *Sexuals*

These are functionally diploid without abnormalities of meiosis (text-fig. 7*a* ; plate I, fig. 3).

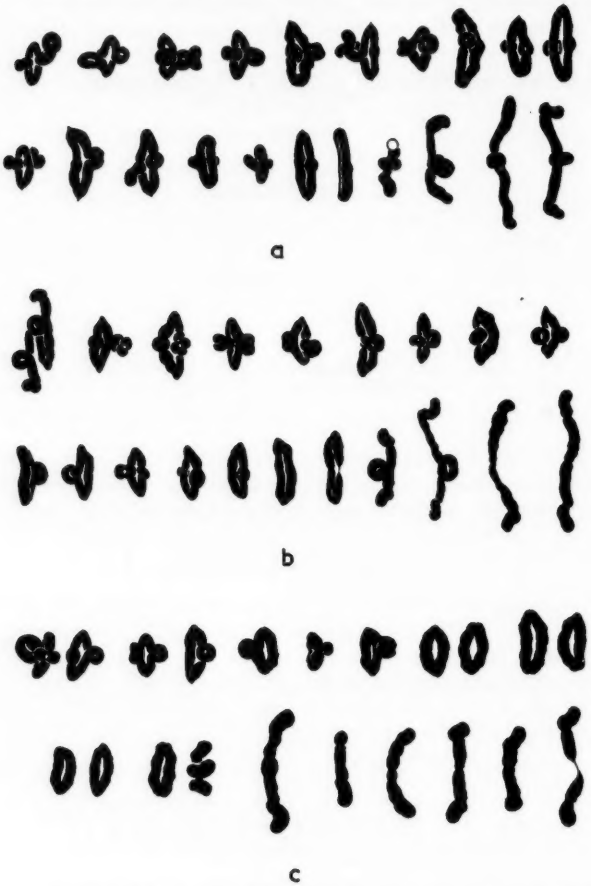
B : *Facultative apomicts*

B1 and B2 ($2n = 42, 43$). Most P.M.C. showed a trivalent or a quadrivalent, occasionally both, at M I, as well as unpaired chromosomes (table 10 ; text-fig. 7*b*). The univalents divide at either the first

* In this species, Hakansson (1943, 1944) found very strong growth of the antipodals in unfertilised flowers. Micropylar groups were regarded as the transformed cells of the egg-apparatus.

or the second division, and their irregular distribution, combined with the unequal separation in trivalents, no doubt result in a proportion of aneuploid progeny with reduced competitive power.

B3 ($2n = 63$). A typical M I had 6 I + 6 II + 15 III and the high



TEXT-FIG. 7.—M I in P.M.C. of hexaploids : complements arranged from left to right in decreasing order of number of chiasmata per bivalent. × 1600.

(*a*) Sexual : 21II (A).
(*b*) Facultative apomict : 1IV 19II (B1).
(*c*) Predominant apomict : 21II (C1).

Note : the obligate apomict with little pairing is shown in plate I, fig. 4.

frequency of trivalents supports the view that the " triploids " arose semi-sexually, that is from failure of meiosis on one side. Quadrivalents and quinquevalents also occurred. Table 11 summarises the frequency of lagging chromosomes and of micronuclei in 373 cells.

The amount of elimination is low. Many A I laggards are presumably included in the telophase nuclei or are able to rejoin the main body of chromosomes at M II. The residue, appearing as micronuclei in the tetrads, are probably lost. But we may expect gametic numbers

TABLE 10

Chromosome associations in P.M.C. of the three kinds of apomicts

Population	2n	Mean frequency per cell of				Total cells
		I	II	III	IV	
B : Facultative :						
1 . . .	42	0·5	19·0	0·4	0·6	*19*
2 . . .	43	1·6	18·9	0·6	0·5	*26*
C : Predominant :						
1 . . .	42	1·6	20·1	0	0·05	*21*
2 . . .	57	6·3	12·6	8·5	0	*10*
3 . . .	63	5·5	7·0	14·5	0	*2*
D : Obligatory :						
1 . . .	42	37·0	2·5	0	...	*181*
1 . . .	42	35·8	3·1	0·013	...	*75*
1 . . .	41	37·9	1·5	0·007	...	*108*

of about 63/2. The chromosome numbers of all five sexual daughter-plants confirmed this expectation (table 1).

The occurrence of multivalents in plants which are numerically polyploid (6*x*) but on the whole functionally diploid, can be taken

TABLE 11

Frequencies of laggards in the "triploid" B3, showing their incorporation in the main nuclei as meiosis proceeds

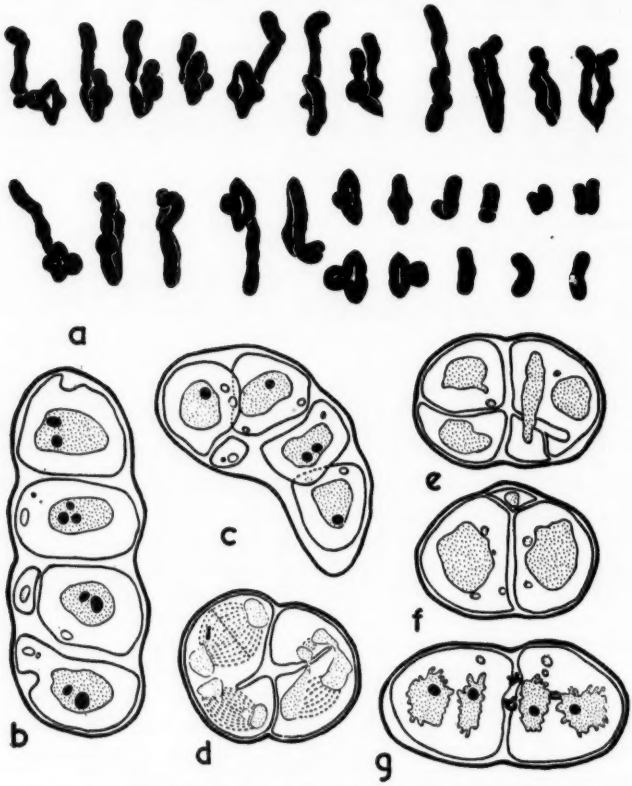
No. of cells	Nos. of laggards or micro-nuclei per cell										Mean
	0	1	2	3	4	5	6	7	8	9	
Late A I . . .	4	12	16	16	11	5	2	1	0	1	*4·7*
Interphase . . .	29	44	37	16	6	1	...	...	...	...	*3·0*
Late A II . . .	77	23	14	2	...	...	...	...	...	...	*1·2*
Tetrads . . .	33	17	4	2	...	...	...	...	...	...	*1·1*

as evidence of pairing between supposed ancestral haploid sets, or of interchange hybridity. The latter is favoured by the fact that apomictic populations will maintain or even increase whatever structural or genic hybridity may have been present in their ancestors.

C : *Predominant apomicts*

C1 ($2n = 42$). This plant had 18-21 pairs at M I (text-fig. 7*c*) and 1-3 pairs of univalents occurred in half the P.M.C. Very rarely a single quadrivalent was found.

C2 and 3 ($2n = 57, 63$). The extra chromosomes were mainly found in trivalent associations (text-fig. 8*a*), confirming their presumed



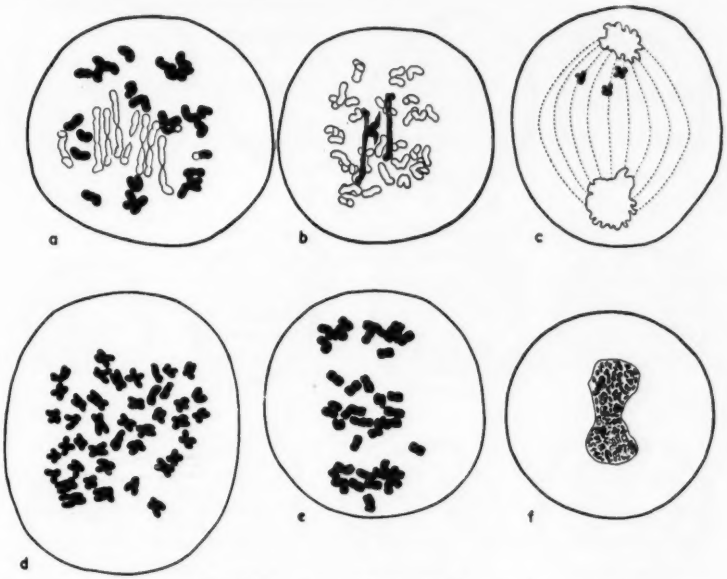
TEXT-FIG. 8.—Meiosis in the P.M.C. of the predominant apomict C3 ($2n = 63$).
(*a*) M I. 16III 4II 7 I. ×1600.
(*b*) to (*e*), (*g*) Irregular tetrads showing micronuclei, microcytes and lagging chromosomes. ×600.
(*f*) End of first division : 6 micronuclei and a microcyte. ×600.

maternal origin. The two meiotic divisions were very variable. At one extreme, the distribution of chromosomes at A I was almost regular, the probable result being functional male gametes, and, as we saw (table 1), occasional fertilisation. But at the other extreme, the range of abnormalities was endless (text-fig. 8*b-g*), and the prospect of balanced pollen grains remote.

D : *Obligate apomicts*

These plants were partly or completely asynaptic and the few bivalents formed had usually a single terminal chiasma (table 10).

Pachytene pairing was apparently complete, but it was probably incomplete in some chromosome pairs, as revealed at later prophase stages. Later association was of three kinds : by chiasmata ; by relational coiling, surviving from pachytene ; and by the parallel alignment of homologues at diakinesis without visible evidence of chiasmata or relics of relational coiling.



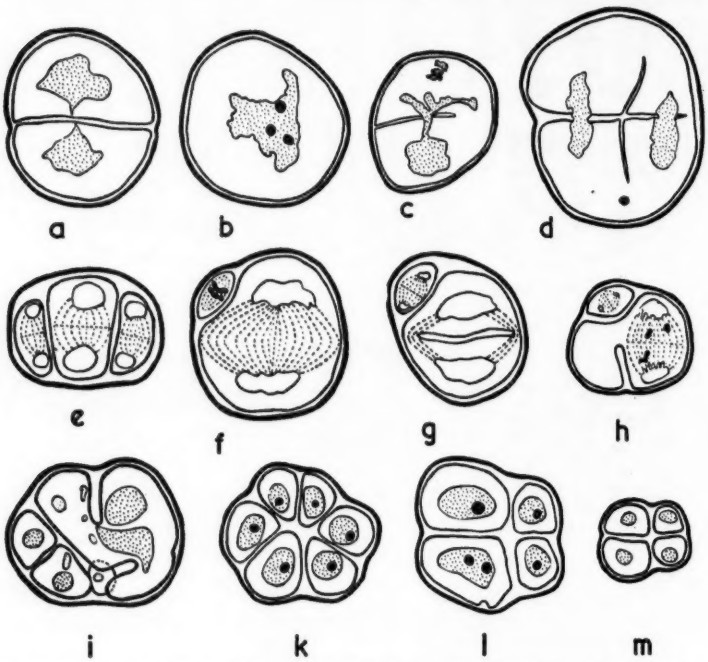
TEXT-FIG. 9.—Meiosis in P.M.C. of two obligate apomicts, D1 ($2n = 41, 42$). ×1000.
(*a*) M I showing 8II 26I.
(*b*) M I with 2II 36I and one neocentric univalent.
(*c*) Late A I with 3 lagging univalents.
(*d*) A I : 32 scattered split univalents, prior to restitution.
(*e*) A I : 42 univalents, concentrated at equator and poles.
(*f*) Restitution nucleus showing halves of split univalents still in contact.

The view that partial or complete failure of metaphase pairing is due to reduced precocity of the prophase is supported by the observations that metaphase bivalents are attenuated or semi-mitotic. This lack of spiralisation is proportional to the degree of failure of pairing (*cf.* text-fig. 9*a-b*, and plate I, fig. 4). But distinction between the obligate apomicts which are asynaptic and the groups of sexuals, facultative and predominant apomicts with nearly complete pairing under similar conditions is no doubt under genotypic control as in parallel experimental cases.

The consequences of variable asynapsis were themselves exceedingly

variable. There was, in fact, only one constant result: the unpaired chromosomes did not effectively divide at the first division (text-fig. 9*c*). The separation of half-univalents was delayed till the second division or its equivalent following restitution. This course was invariable.

The appearance of doubleness, which affected all univalents in the nucleus simultaneously, was the only reliable criterion, at least in completely asynaptic cells, of the development of the cell. Variation in the dispersal of univalents, whether they were scattered (text-fig. 9*d*)



TEXT-FIG. 10.—Meiosis in P.M.C. of two obligate apomicts, D1 ($2n = 41, 42$). ×550.

(*a*) First division successful (although a bridge has been broken).
(*b*), (*c*) First division suppressed giving restitution nuclei.
(*d*) Restitution at second division.
(*e*) Triad of cells.
(*f*) to (*h*) Division following partial restitution; chromosome or chromosomes left out have formed micronuclei which have divided.
(*i*) to (*m*) Irregular and uneven products of a two-division meiosis.

or apparently more orderly (text-fig. 9*e*), was found equally at metaphase or at anaphase. Hence the transition from metaphase to anaphase could not be described in terms of chromosome movements.

Movement to the equator of the cell, or congression, since it was largely a movement of unpaired chromosomes, was much delayed in the obligate apomicts, reaching its extreme in cells with no bivalents at all. The remote position from the equator of many univalents

indicates that probably they had not moved at all during prometaphase. In these cells, two consequences followed :

(*a*) *Restitution* occurred because the chromosomes were so out of step in relation to the development of the spindle that they had time to split but no time to separate and move to the poles (text-fig. 9*d-e*, text-fig. 10*b-c*). All chromosomes, or nearly all, were therefore included in a common restitution nucleus (text-fig. 9*f*) and this was succeeded by a single division giving dyads and unreduced pollen grains. Individual chromosomes, or small groups, left out of the main nucleus, formed micronuclei or microcytes (text-fig. 10*c*, *f-h*).

In some anthers, restitution probably occurred at prometaphase or even earlier. The nuclei were then rounded and compact (plate I, figs. 5-6) and sometimes their contents gave the false impression that they were degenerating. These figures closely resemble the " contraction nuclei " described by Fagerlind (1947*a*, *b*) in male *Hieracium* and female *Erigeron*.

TABLE 12

The potential products of meiosis in P.M.C. : variation within one spikelet of the obligate apomict D1

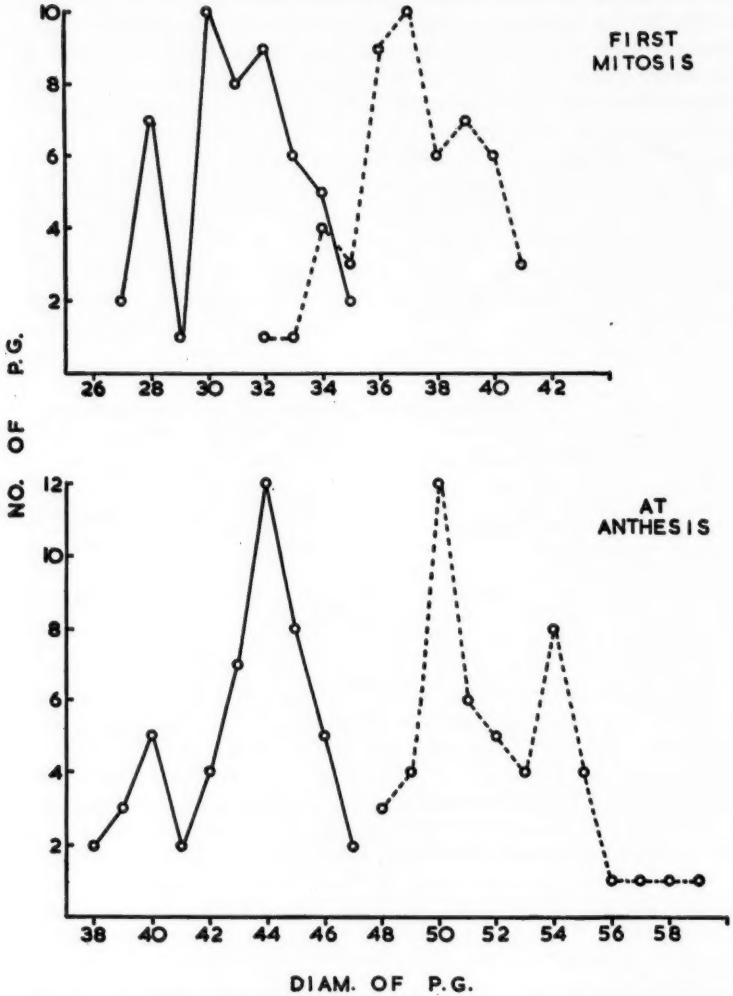
6*x* sample	Total P.M.C.	Restitution after A I			Total potential P.G.	Estimated ♂ gametes per cent.		
		Complete	Partial	None		"Diploid" 6*x*	"Hypodip" 6*x*—	"Haploid" ±3*x*
1	**124**	9	27	88	*424*	4·2	12·8	83·0
2-3	**81**	72	...	9	*180*	80·0	0	20·0

(*b*) Cell-division sometimes occurred, owing to a bipolar grouping of unsplit and static univalents which did not interfere with wall-formation. The result was sometimes a triad of cells (text-fig. 10*e*).

Neocentrics. P.M.C. with 1-2 bivalents were the more instructive since the bivalents served as reliable markers of the state of the nucleus. In one plant, a 2*n*—1 aberrant arising in D1, co-orientation of the bivalents revealed an interesting abnormality (text-fig. 9*b*). One of the 37 univalents in this cell (and in eight further metaphases) was oriented like a bivalent owing to neocentric activity at its two ends (*cf.* Rhoades and Vilkomerson, 1942 ; Prakken and Müntzing, 1942, and Ostergren and Prakken, 1946).

Two-division meiosis. When 3-8 bivalents were formed, the usual result was an irregular meiosis giving tetrads and reduced but very unbalanced pollen grains. Both chromosome distribution and cell division were uneven (text-fig. 10*i-m*), and complicated by the lagging chromosomes, micronuclei and microcytes. Lagging univalents dividing at A II sometimes promoted restitution in one or both cells (text-fig. 10*d*).

Pollen grains. For present purposes, the results of meiosis in terms of the kinds of male gametes produced are of some importance. The expectations in obligate apomicts were approximately estimated by



TEXT-FIG. 11.—Graphs showing diameters in microns of reduced pollen ($3x$) from the sexual population A ($2n = 42$) and unreduced pollen ($6x$) from the obligate apomict D2 ($2n = 43$).

scoring the numbers of restitution nuclei and the recognisable products of restitution ; and also the stages, M II to tetrads, which could be related to a two-division meiosis. Three samples of cells were analysed,

one from each of the three buds of one spikelet fixed at the same time. The results were radically different (table 12).

Were gametes of these several kinds produced? This information, though not available in the same plant, was obtained from pollen grain mitoses in a closely related plant (ex D2) with 43 chromosomes. All 50 mitoses scored had 43 chromosomes or approximately so. On the other hand, no divisions at all were seen in the considerable range of smaller, usually empty and abortive grains which were distributed at random amongst the unreduced grains in any one loculus. Viable pollen grains were therefore formed. But they were predominantly, or exclusively, unreduced.

This conclusion was strengthened in another way: by measurements of known reduced (sexual) and unreduced (apomictic) pollen grains at mitosis, and at maturity. Both sets of data had the same result (text-fig. 11): in the apomict, grains were twice as large as in the sexual. We must therefore conclude that the unreduced and usually more balanced products of restitution and dyad-formation have an immediate selective advantage over the reduced and usually unbalanced segregants of an irregular meiosis, the majority of which do not survive. In a population where the pairing of chromosomes has become slightly defective it is advantageous that it should evolve towards being totally defective.

Finally, what is the function of the pollen in the obligate apomicts? One potential role is external and depends upon the ability of the pollen to function in crosses with sexual species. The second task of the pollen is internal and indispensable: it is pseudogamy. The nature of this process will now be considered.

9. PSEUDOGAMY

Hitherto the term has been used in a dual sense, to denote a stimulating effect by the pollen entering the style upon embryo and endosperm development or to indicate fertilisation of the polar fusion-nucleus. In most examples adequately investigated, the more positive interpretation is valid, and it is perhaps universally true. The crucial test is the determination of chromosome number in the endosperm, as shown so elegantly by Rutishauser (1955).

In *Agropyron*, counts were preferably made in young, free-nucleate endosperm which was multiplying rapidly. Older cellular tissue had fewer divisions and an increasing tendency for endomitosis and other irregularities. The best period was at 48-96 hours after pollination.

Counts were confined to a "selfed" obligate apomict (ex D2) with 43 chromosomes, the self-same plant in which only unreduced pollen grains were found. It had a relatively "hexaploid" endosperm with $6n = circa\ 128$. This showed, as we supposed, that "diploid" pollen is favoured by selection in this plant, and that, as we now find, its primary function is fertilisation of the polar nuclei. Following

Darlington (1952), we may distinguish the established cases of pseudogamy through triple fusion as *paramictic*.

Returning to the crossing experiments in table 2 we may recall that sexual octoploids gave better results than hexaploids or tetraploids in pollinating the obligate apomicts. Evidently this was because the apomict is accustomed to hexaploid pollen which the sexual octoploid most nearly approaches.

10. THE BREAKDOWN OF SEXUAL REPRODUCTION

Different wild populations of one and the same polymorphic species we now see in successive stages of the breakdown of sexual reproduction. This breakdown culminates in a stable system of obligate apomixis. Such a sequence has hitherto been demonstrated in *Poa alpina* (Müntzing, 1933, 1940). *Agropyron*, however, has certain entirely new features.

(*a*) All the New Zealand species of *Agropyron scabrum* are self-fertilising ; they belong to the important wheat and barley group where self-fertility is widespread and where apomixis has not previously been found.

(*b*) No natural hybrids within or between the various New Zealand species have been found.

(*c*) There is a gradual reduction in chiasma frequency which finally results in the almost complete failure of chromosome pairing found on both male and female sides in the obligate apomicts (*cf.* text-figs. 7 and 9).

The first step in the development of apomixis from sexual reproduction is the occurrence of restitution nuclei. These in a self-fertilising species presumably arise from genes leading to failure of pairing, or from " triploidy " (which in any case is likely to follow). The consequence—facultative apomixis—is therefore not due to hybridisation but to new genotypes arising from inbreeding or mutation. Types of " mutation " that would be effective in this way, such as deficiencies and dicentrics, we see later appearing in these systems, predominantly or habitually apomictic, which favour both their origin and their persistence.

The present nature, and the future trend of our facultative population (table 1), is indicated by the breeding structure of its components, as follows :

(i) Facultatively parthenogenetic " haploids " ($3x$).

(ii) Sexual " diploids " ($6x$) whose subsequent evolution may be either sexual by return to the normal diploid pool, or subsexual.

(iii) Semi-sexual " triploids " ($9x$), arising by fertilisation of restitution eggs, and immediately capable of obligate parthenogenesis though occurring in a facultative population. As in the diploids, their further evolution is subsexual.

We shall see later what the subsexual process is and how it occurs.

Two conditions, and their coincidence, are now necessary for the genesis of habitual apomixis, *viz.* :

(*a*) Regular suppression of meiosis by the segregation and selection of genetic types having this effect.

(*b*) Regular omission of fertilisation equally known to arise under genotypic control.

These conditions have largely been found in the predominant apomicts. In the " diploids ", the rare meiosis is abortive : the suppressed meiosis occasionally results in subsexual recombinants. In " triploids ", semi-sexual reproduction is rare, and in these higher chromosome classes the failure of female reduction has mainly been secured by a complete changeover from a suppressed meiosis to mitosis. We can therefore regard the segregate with 94 chromosomes (C_4) as an obligate apomict arising from its predominantly apomictic parent (C_3).

The final phase in the succession has been achieved by the obligate apomicts. Here, in all instances where the embryo-sac originates purely by mitosis, sexual reproduction, in the genetic sense, has been completely suppressed. But where the embryo-sac arises by a suppression of meiosis giving dyad formation, the restitution eggs arising in dyads show, and will always show, genetic recombination of the kind called *subsexual reproduction* (Darlington, 1937, 1939).

Subsexual recombination, as shown by Darlington, will explain " mutation " in apomicts, and the recombination of its results, both arising through crossing-over in the unreduced E.M.C. Our system of diploid parthenogenesis is therefore subject to variation by the same means as its sexual ancestors expect that the recombinations are now at a subsexual level : they no longer have to pass through a haploid sieve and therefore permit the more violent structural and numerical changes which we have actually found.

Analysis in all our apomictic groups has been simplified by the absence of apospory, revealing the crucial facts of competition between ovules whose products in the beginning are meiotic and sexual, but later subsexual and purely non-sexual. This succession is a female succession. There is also, however, a male succession. All classes of apomicts retain their male-sexuality, the opposite effect to male-sterility. This ensures the production of some viable pollen, capable of fulfilling its pseudogamous function. Good pollen still arises by reduction in the predominantly apomictic plants but by non-reduction in the habitual apomicts. Parallel genetic changes have produced parallel adaptive results on the two sides.

The morphological concomitants of the system described—a system of diploid parthenogenesis combined with subsexual recombination—have all been seen. Observations of mitosis, meiosis,

morphology, and the breeding system all agree in showing how the subsexual system works. The obligatory apomict shows how it works and how it evolves since it is the last development in the whole irreversible process.

11. SUMMARY

1. In the New Zealand grass *Agropyron scabrum* four populations A, B, C and D, all basically hexaploid, were found to differ in mode of reproduction. Representative plants and their seedlings to the number of 999 were compared in :—

(i) chromosome complements ;
(ii) behaviour at meiosis in E.M.C. and P.M.C. ;
(iii) development of embryo, endosperm and seed ;
(iv) systematic character.

These modes of study gave correlated results in the four populations as follows :

A. Completely and normally sexual : uniform in chromosome complement and in systematic character.

B. Facultatively apomictic : meiosis and fertilisation both liable to fail but in an uncoordinated way with production of " haploids ", " triploids " and unbalanced forms. Such a system may be said to be versatile, that is sexual, asexual and semi-sexual. The population is, as expected, highly variable in fertility and in systematic character.

C. Predominantly apomictic : meiosis largely suppressed on the female but not on the male side. Different chromosome types giving a limited stereotyped variation.

D. Obligatorily apomictic : suppression of reduction on male and female sides ; loss, gain and breakage of chromosomes in 5 per cent. of the experimental progeny but by elimination great constancy of the natural population.

2. In the three apomictic populations B, C and D :

(i) Embryo-sacs arise from mother-cells in whose meiosis reduction is suppressed but crossing-over and other chromosome changes derived from it still occur. Such reproduction is described as subsexual.

(ii) The embryo depends for its development on that of the endosperm which in turn depends on fertilisation of the fusion nucleus by a male generative nucleus. In B and C reduced pollen is chiefly effective ; in D unreduced pollen.

3. The difference between the suppression of pairing at meiosis on male and female sides in population C (and to a less extent D) shows that genotypic control, not hybridity, is responsible for the suppression of chromosome pairing in them.

Plate I

FIGS. 1-2.—Mitosis. × 1400.

FIG. 1.—" Triploid " ($9x = 63$) predominant apomict C3. Six nucleolar chromosomes.

FIG. 2.—" Haploid " ($3x = 21$) facultative apomict B1.

FIGS. 3-6.—Meiosis in P.M.C.

FIG. 3.—M I with 21II. Sexual population (A). × 1800.

FIG. 4.—M I with 1II 40I. Obligate apomict (D1). × 1200.

FIGS. 5-6.—Two-division and one-division meiosis in the same anther of the obligate apomict (D1). × 300.

FIG. 5.—Restitution, together with typical M II.

FIG. 6.—Restitution and the equivalent M II following it.

2

4

6

Plate II

Early development of the embryo-sac in sexual (A) and apomictic (C, D) populations. Figs. 1-5 ×800 ; fig. 6 ×160.

FIG. 1.—Meiosis in the E.M.C., pachytene, *cf.* fig. 3 below (A).

FIGS. 3, 5.—Mitosis in the E.M.C.

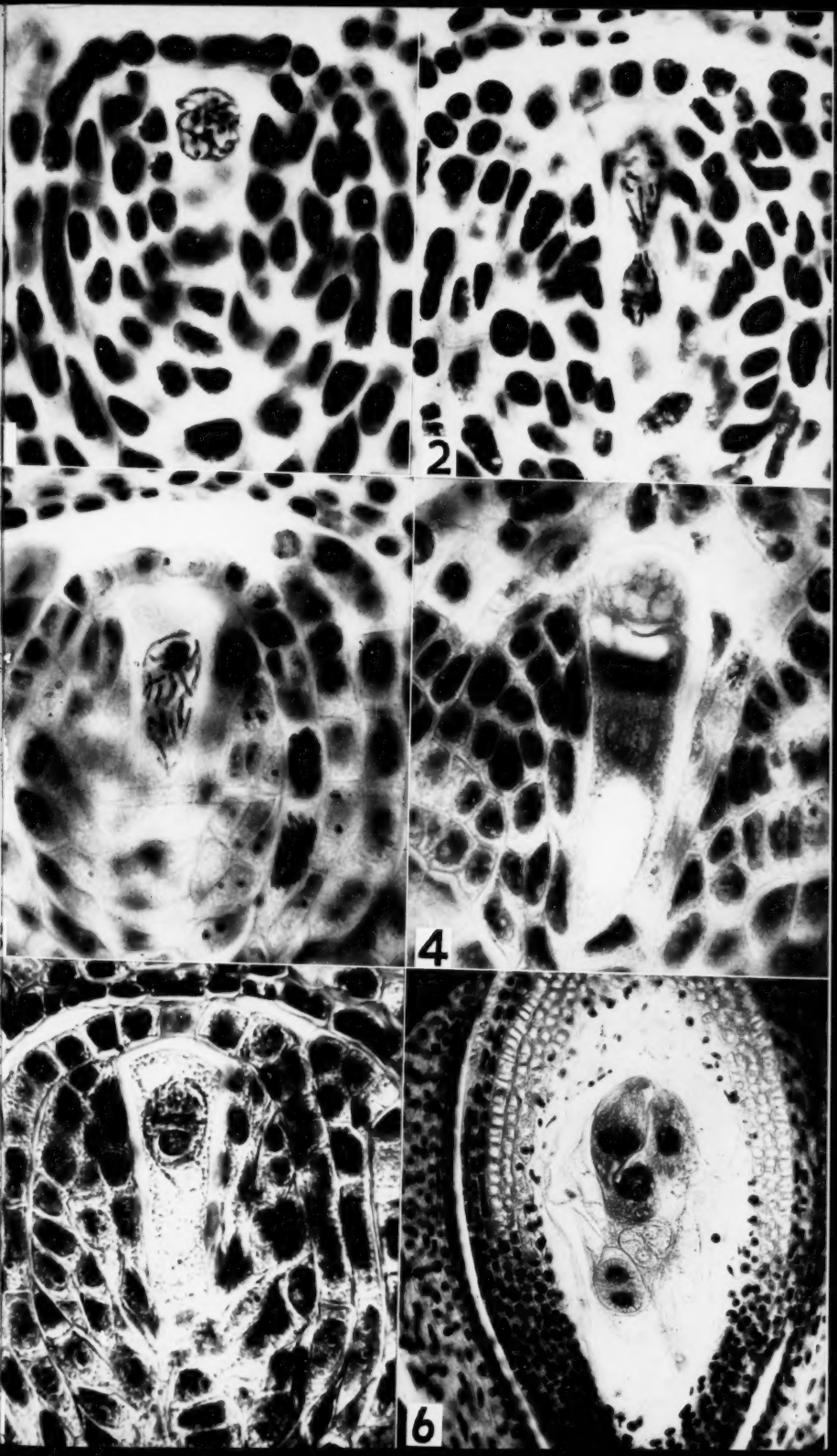
FIG. 3.—Late prophase (D_4).

FIG. 5.—Vacuolate resting stage (C_1).

FIG. 2.—Restitution in E.M.C. of C_1.

FIG. 4.—2-nucleate E.S. showing remnant of micropylar cell of dyad (C_3).

FIG. 6.—Precocious two-celled embryo at the time of anthesis (C_3).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4. The stages in the evolution of apomixis are complete in this species except for the absence of exclusively mitotic types. They appear in their simplest succession because of the strict inbreeding of all populations and the absence of the complication of apospory. Subsexual recombination provides for variation in the genotypic control of the reproductive mechanism and hence for adaptive evolution of the genetic system.

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ABO BLOOD GROUPS AND RACIAL CHARACTERISTICS IN RURAL WALES

I. MORGAN WATKIN

County Health Department, Aberystwyth, formerly of the John Innes Institution and National Blood Transfusion Service, Wales

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1. INTRODUCTION

PROOF of the inheritance of the *ABO* blood groups was followed by the studies of L. and H. Hirschfeld who showed that the frequencies of the groups varied in different peoples. Many investigations of these groups have since been published. For example, Vérzar and Weszeczky found that the *ABO* groups of Hungarian gypsies differed from those of the Hungarians among whom they lived but was almost identical with that of the natives of North-West India. This origin had already been ascribed to the gypsies on other grounds.

The blood group analysis in Wales of Boyd and Boyd in 1937 related to fewer than 200 persons whilst the survey of Fraser Roberts in 1942 was confined to the North Wales counties of Caernarvon, Denbigh and Flint. The campaign undertaken during the last war resulted in extensive bleeding and grouping. An analysis of these findings has been made to ascertain whether the Welsh people are an assembly of many racial stocks, each of which has intermingled relatively little with the other, as has been suggested by physical anthropologists, or whether, on the other hand, the population is now a genetically homogeneous whole. An attempt has also been made to correlate the results in Wales with those obtained elsewhere in the Old World in the hope that light may be thrown on the history of human settlement in the Principality. An account of the preliminary findings and their relation to language in particular was given by Mourant and Watkin in 1952.

A brief account of the ethnology of the Welsh people, based on the views of physical anthropologists and historians, is an essential pre-requisite to a study of the serological differences observed in Wales.

2. THE HUMAN HISTORY OF WALES

Stone Age Man. Remains of early man before he took to cave dwelling have not been discovered in Wales and Wheeler dates the first immigration between 15,000 and 10,000 B.C. Fleure and James in their study of anthropological types in Wales maintain that along moorlands such as the Black Mountain, Carmarthenshire and Mynydd Hiraethog, in West Denbighshire, human types closely resembling Palæolithic groups still persist.

The human type associated with the Neolithic immigrations commencing about 2500 B.C. was long-headed, of medium height and probably had dark hair. A similar type is found in considerable numbers in the coal-mining valleys of South Wales, along the moorlands of South Cardiganshire, North Pembrokeshire and North Carmarthenshire. This has led Fleure and James to state that the long-headed brunet is the fundamental type in Wales and that it dates back to early Neolithic times.

Beaker and Early Iron Age Folk. The general distribution of beakers in Wales is consistent with an eastern origin. Colonisation of South Wales occurred by land from the Upper Thames-Cotswold area and by sea from Somerset. North Wales was probably reached by sea from Westmorland whilst the Upper Severn Valley was the main gateway into Mid-Wales.

The physical form of the Beaker people varied, those found at Merthyr Mawr, Glamorgan, being of medium height and broad-headed. Their colouring was probably fair and Beddoe's observation that, of a substantial number of broad-headed men examined in the West of England and Wales most had hair that was lighter than that of the rest of the population, seems noteworthy.

In a series of migrations from Northern Gaul from 500-50 B.C., the Early Iron Age Folk reached Britain. The Belgæ, described by Strabo as fair in colour and six inches taller than the tallest men in Rome, were the last to arrive and did not reach Wales until the beginning of the first century A.D.

On the eve of the Roman conquest Wales appears to have been composed of four main tribal divisions :

(1) The modern counties of Monmouth, Brecknock and Glamorgan inhabited by the Silures, a dark-haired people ;

(2) Present-day Carmarthenshire, Pembrokeshire and Cardiganshire in the possession of the Demetae, a tribe apparently related to the Silures ;

(3) North-West Wales held by the Venedotae while the intervening area, comprising present-day Montgomeryshire and Radnorshire together with parts of Merionethshire and Denbighshire, was peopled by the Ordovices or " hammer men " who were among the very last to be subjugated by Rome ;

(4) The north-eastern corner of Wales inhabited by the Deceangli, regarded by some as related to the Venedotae.

The Romans. The line of demarcation between civil and military areas was usually formed by the base fortresses of the Roman legions. Thus it is only in the south-eastern corner of Wales, to the rear of the second legion at Caerleon-on-Usk that one can expect to find traces of a settled Romanised population on any extensive scale. The only walled Roman town found in Wales is at Caerwent, eight miles to the east of Caerleon. With the exception of South-West Wales, Lleyn and Anglesey, all of which appear to have lain even beyond what Haverfield terms the " military zone ", the remainder of Wales became a network of forts and outposts.

It is generally accepted that although the Romans left an impression on the laws and institutions, on the material civilisation and on the language of the country, their genetic contribution to the synthesis of modern Welshmen was insignificant.

The Déssi and the Men of the North. Contemporaneously with the Roman occupation, landings from Ireland on the Welsh coast assumed increasing proportions and culminated in the settlement in Pembrokeshire in the third century A.D. of an entire Irish tribe, the Déssi. The parts of Wales most affected by Irish settlements were those which project towards Ireland and which, in favourable weather, permit of visibility from coast to coast. These include the peninsula of Lleyn, the isle of Anglesey as well as Pembrokeshire.

Cunedda and his followers left the region around the Solway Firth and made landings from Anglesey to the mouth of the Dee. By the fifth century the " Men of the North " who were Brythons by language had become masters of that part of Wales which lies between the rivers Teifi and Dee.

The Anglo-Saxons. Following the battle of Deorham in A.D. 577, the Anglo-Saxons obtained access to the Bristol Channel and severed the connection between the Britons of Wales and those of the Dumnonian peninsula. Similarly, at the battle of Chester in A.D. 616, a wedge was driven between the Britons of North Wales and those of North-West Britain. From the middle of the seventh century onwards the Welsh people were marked off from the other inhabitants of Britain. The delineation of the frontier between England and Wales was left to Offa in the second half of the eighth century. That the Anglo-Saxons kept to the east of the dyke is shown by the distribution of their burial places and by the linguistic formation of place names ; those to the east being, in the main, English and those to the west being, in the main, Welsh.

Wales received no ethnic contributions of any import from across the dyke. The statements formerly made that the natives of South-Eastern Britain, when driven from their homes by the Saxon invader, found refuge in considerable numbers in the mountains of Wales and thus became the ancestors of the Welsh people, have no foundation in fact.

The Vikings, Normans and Flemings. While many parts of Wales felt the effect of Norse raids, the extent of permanent settlements is a matter of dispute. It is believed that, in Pembrokeshire, both sides of Milford Haven were occupied at an early date and that this settlement extended inland as far as Narberth.

Wales received the attention of almost the entire Norman army in Britain and by the end of the twelfth century most Welsh noble families were connected with the French by ties of blood. Unions between victor and vanquished were greatest in the garrison towns and least in the heart of the country where very few, if any, occurred.

The Flemings were transferred by Henry I from the North of

England to South Pembrokeshire where it was thought they would form a useful bridgehead against the Welsh. That part of Pembrokeshire which was occupied by the Flemings, on the one hand, and probably by Vikings, on the other, is known as "Little England beyond Wales".

The Industrial Revolution. After the Norman Conquest, Wales escaped the advent of immigrants on a noteworthy scale until the establishment of the iron and coal industries in the latter part of the eighteenth and nineteenth centuries. Of the 1,120,910 persons enumerated in Glamorgan at the 1911 Census, 390,941 originated from outside the county. A large proportion came from Gloucestershire and Somerset. A similar state of affairs was found to exist in Monmouthshire.

3. TECHNIQUE

Owing to the large number of persons evacuated to Wales during the last war, the donor panel in rural areas contained a far from negligible proportion of non-Welsh persons. To overcome this difficulty the "surname technique"—a technique based on a separation of donors into those with Welsh and those with non-Welsh family names—was used. By this method Fisher and Vaughan (1939) had been able to demonstrate a significant difference in the *ABO* blood group frequencies of the English and Welsh elements at Slough. In the present survey only persons with Welsh surnames have been studied with the exception of Chester and Malpas where figures for English surnames are given.

(i) *Welsh surnames*

From the time of the Welsh Princes to the Tudor period it was a common custom for Welshmen of all walks of life to have their pedigrees, up to the ninth generation, embodied in their ordinary name for juridicial purposes. The following affords an example: Llewelyn ap Dafydd ap Ieuan ap Griffith ap Meredith ap Eynon ap Morgan ap Owen ap Llywarch. Such a cumbrous name was not adapted for a long life and would eventually become curtailed to Llewelyn ap David (or Dafydd). The father's Christian name thus became his son's surname and the abbreviations *ap* or *ab* (meaning "son of") were either dropped or become absorbed in the parent's baptismal name to yield such forms as: Bevan, Bowen, Parry, Price, Pugh. "This system of changing surnames each generation flourished in certain parts of Wales until the middle of last century or later, when the then existing surname became permanent, with the result that the last paternal baptismal name now became, once and for all, the family name". "Another feature about baptismal names used as surnames", writes Morris, "is that the clergy and members of the official classes added the letter *s* (presumably meaning *son*, or marking the possessive case) to the baptismal name when it was

used as a surname ; thus John becomes Johns and Johnes and finally Jones ".

The late Canon C. W. Bardsley in his examination of surnames in Western Europe draws attention to the unusual conditions prevailing in Wales. He writes as follows : " Wales is the great exception. Here there is scarcely a trade name, only a few nicknames, no official surnames that I know of, just a sprinkling of local surnames, and the rest quite 95 per cent. are baptismal names. Hence the great difficulty of identification in the Principality ".

Appendix (f) shows a list of surnames which I have taken to be Welsh. Where a particular name is to be found both in England and Wales, and where there is no proof of its exclusively Welsh origin, the country in which it is the commoner will be regarded as its home. For this latter purpose H. B. Guppy's *The Homes of Family Names* has been used.

(ii) *Selection of areas*

A substantial part of the rural population in Wales lives in scattered homesteads—a state of affairs dissimilar to that found in England where the village movement is very strong. Furthermore, only one county in England, Westmorland, is as thinly populated as the rural counties of Mid and North Wales. The areas selected for study must, therefore, be sufficiently large to ensure statistically sound comparisons. The physical configuration of Wales permits of such divisions in the form of moorlands, broad valleys, coastal plains and peninsulæ.

(iii) *Blood group determination*

Every blood sample was grouped by the National Blood Transfusion Service on both cells and serum. The grouping was re-checked each time a person donated blood.

(iv) *Calculations*

Group frequencies, gene frequencies, the expected percentage of group *AB* and a Chi-square test for homogeneity have been calculated for the principal regions, for each individual area and for each township having a sample of not less than a hundred. Fisher's method has been used in calculating gene frequencies. A comprehensive list of towns and villages which make up each area, together with the numbers belonging to each *ABO* blood group, will be found in appendices (a-e).

4. ABO BLOOD GROUPS

(i) *General*

There is a tendency for blood group analyses, involving large numbers, to show a deficiency in group *AB*. As donors do not always resign in equal proportions from each of the four groups, it is important that investigations be based on the combined active and resigned

panels. If this is carried out, a deficiency in group *AB* may, with more confidence, be ascribed to errors in grouping. It is satisfactory to record, however, that the Welsh sample does not show a deficiency in group *AB*, *vide* table 1.

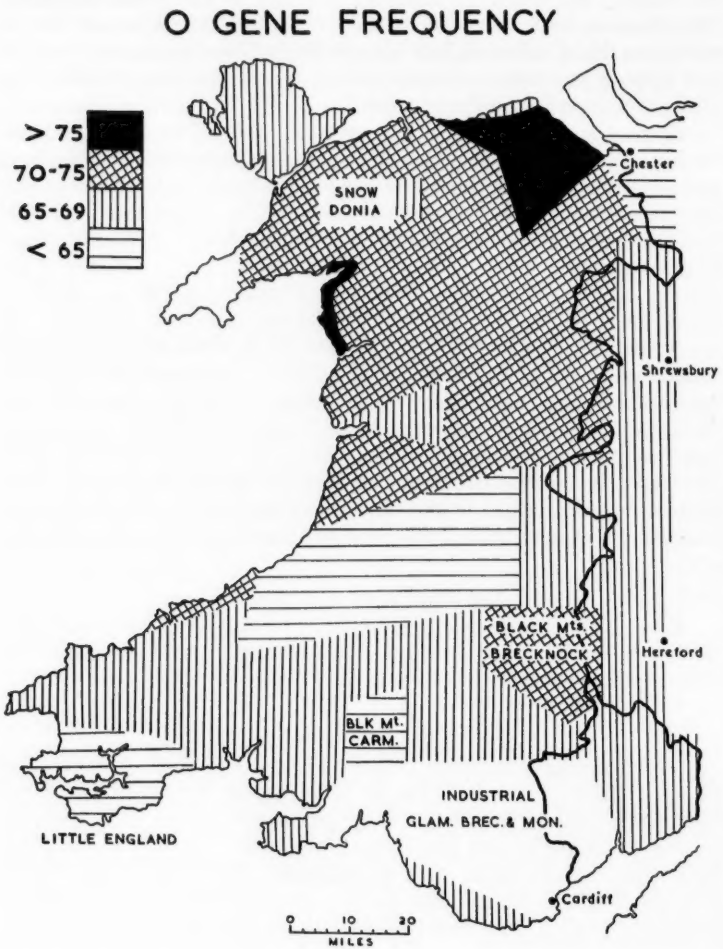

Fig. 1.

In this table Wales has been divided, for comparative purposes, into three regions, north, mid and south. The north consists of the counties of Anglesey, Caernarvon, Denbigh, Flint and Merioneth, whilst Mid-Wales is made up of Montgomeryshire, Radnorshire, North Cardiganshire and North Brecknock. The southern region

comprises the remainder of Wales other than the Glamorgan and Monmouthshire coalfields which, owing to their known heterogeneity, are a problem unto themselves.

The salient feature is the rise in *O* gene frequencies, ultimately reaching 72 per cent., as one proceeds northwards. There is a parallel fall in the *A* frequency. The rise is not gradual but takes place in abrupt steps, the principal one lying near the Upper Severn Valley

TABLE 1

The rise in O *gene frequencies as one proceeds northwards*

Region	Total	Group frequencies				Expected *AB* per cent.	χ^2 for homogeneity	Gene frequencies		
		O	*A*	*B*	*AB*			*O*	*A*	*B*
North Wales .	4535	52·0	34·9	10·2	3·0	2·8	*0·29*	72·2	21·1	6·7
Mid-Wales .	5088	49·3	38·1	9·5	3·1	3·0	*0·15*	70·3	23·3	6·5
South Wales .	7137	45·6	40·4	10·3	3·6	3·7	*0·00* (4)	67·6	25·2	7·2
Total . .	16,760	48·5	38·2	10·0	3·3	3·2	0·22	69·6	23·5	6·9

in Montgomeryshire, *vide* fig. 1. A rise in *O* as one proceeds northwards is also found in England, but, at similar latitudes, *O* is higher in Wales than to the east of Offa's dyke. South Wales is higher in *O* than Gloucestershire, whilst North Wales has *O* frequencies at least as high as those found in Northumberland.

TABLE 2

The variation in the B *gene frequency*

Region	Total	Group frequencies				Expected *AB* per cent.	χ^2 for homogeneity	Gene frequencies		
		O	*A*	*B*	*AB*			*O*	*A*	*B*
Western Wales .	8632	47·0	38·4	10·9	3·7	3·6	*0·12*	68·5	23·9	7·6
Eastern Wales .	8128	50·1	37·9	9·1	2·9	2·8	*0·03*	70·8	23·1	6·2

Note.—A fourfold table for $B+AB$: $O+A$ in Western Wales versus $B+AB$: $O+A$ in Eastern Wales gives a $\chi^2 = 25{\cdot}42$. For one degree of freedom $P < 0{\cdot}00001$. The difference is highly significant.

If Wales is divided into eastern and western halves, the latter being made up of Anglesey, Caernarvonshire, Merionethshire, the Dyfi Basin of Montgomeryshire, Cardiganshire, Pembrokeshire and Carmarthenshire, one observes that *B* is significantly higher in Western than in Eastern Wales, *vide* table 2. This is the reverse of that found in Europe generally where *B* is highest in the east and falls as one proceeds westwards.

This general picture, however, conceals a number of significant local variations which will be discussed later.

(ii) *Areas of high O frequency*

North Wales. Fig. 1 reveals that most of North and Mid-Wales have *O* gene frequencies exceeding 70 per cent. In some parts of North Wales frequencies as high as 75 per cent. were noted—an observation similar to that made by Fraser Roberts. Inspection of table 3 reveals that the Ruabon-Ruthin moorlands and the Middle Dee Valley, the Bala Cleft and its branches, Ardudwy Mountain and the South Caernarvonshire coastal plain have lower *O* frequencies

TABLE 3

The areas of high O gene frequency

Area	Total	Group frequencies				Expected *AB* per cent.	χ^2 for homogeneity	Gene frequencies		
		O	*A*	*B*	*AB*			*O*	*A*	*B*
North Caernarvonshire coastal plain	459	54·7	34·9	8·1	2·4	2·2	*0·08*	74·0	20·7	5·3
South Caernarvonshire coastal plain	350	50·0	36·3	10·6	3·1	3·2	*0·00(02)*	70·7	22·2	7·1
Coastal plain of Ardudwy	550	55·8	29·8	10·7	3·6	2·4	*2·43*	75·2	17·9	6·9
Ardudwy Mountain	277	49·8	35·0	12·3	2·9	3·5	*0·23*	70·4	21·5	8·2
Denbighshire coastal plain, Clwyd valley and Eastern Flintshire	797	56·8	32·9	8·4	1·9	2·1	*0·12*	75·3	19·3	5·4
Ruabon-Ruthin moorlands and Middle Dee Valley	897	49·3	37·8	10·0	2·9	3·2	*0·13*	70·1	23·1	6·8
Mynydd Hiraethog	181	55·2	28·7	13·8	2·2	3·0	*0·31*	74·0	17·2	8·7
Bala Cleft and its branches	552	50·9	33·5	11·6	4·0	3·2	*0·86*	71·6	20·6	7·7
North Montgomeryshire	636	52·5	35·7	8·0	3·8	2·3	*4·54*	73·0	21·6	5·4
Upper Severn System	2157	51·9	36·1	8·9	3·1	2·6	*1·40*	72·2	21·8	5·9
Aberystwyth-Plynlymon area	487	52·6	35·3	8·6	3·5	2·4	*1·67*	72·9	21·4	5·8
Coastal South Cardiganshire	273	49·8	37·0	9·2	4·0	2·8	*1·05*	71·0	22·7	6·3
Epynt, Black Mountains of Brecknock, Upper Wye and Upper Usk area	1049	51·0	37·1	9·0	3·0	2·7	*0·18*	71·5	22·5	6·0

than the remainder. These four areas form a continuous wedge of territory passing westwards from the English border to Cardigan Bay. It seems as though the very high *O* population had, in these parts, been diluted by another stock appreciably lower in *O* which entered either from England or from the Irish Sea.

Physical anthropologists have shown that the Bala Cleft and the side valley, leading to Trawsfynydd, harbour a proportion of tall men. An appreciable admixture of people must have occurred in these areas for the Cleft was one of the principal gateways from England into Wales. As early as Bronze Age times trade routes from the Western Midlands passed through the Cleft and across Ardudwy Mountain to reach the North-West Wales bays and estuaries.

The Upper Severn System. To the north of the Severn Valley, in Montgomeryshire, the *O* frequency is high, whereas to the south it is significantly lower—an indication of the existence of two rather different peoples. The historical geography of the area offers an explanation of this observation.

The ancient centres of population, as Fleure and Whitehouse have pointed out, were the lightly-wooded or treeless moorlands. The valley sides and valley floors, on the other hand, were woodland or malaria-ridden swamps harbouring ferocious animals against the hazards of which man, with primitive stone tools, was able to achieve

TABLE 4

The frequencies in the towns named in table 3

Town	Total	Group frequencies				Expected *AB* per cent.	χ^2 for homogeneity	Gene frequencies		
		O	*A*	*B*	*AB*			*O*	*A*	*B*
Caernarvon	161	57·8	32·3	8·7	1·2	2·1	*0·42*	75·7	18·8	5·5
Bangor	110	45·5	44·5	8·2	1·8	3·2	*0·45*	67·0	27·3	5·8
Llandudno	130	55·4	33·8	7·7	3·1	2·0	*0·57*	74·8	20·2	5·0
Pwllheli	101	48·5	31·7	14·9	5·0	4·0	*0·18*	70·0	20·0	10·0
Portmadoc	128	47·7	39·1	9·4	3·9	3·1	*0·18*	69·3	24·2	6·5
Penrhyndeudraeth	223	57·4	29·6	9·0	4·0	2·0	*3·60*	76·5	17·7	5·8
Trawsfynydd	166	48·8	35·5	13·3	2·4	3·9	*0·69*	69·3	21·8	8·9
Colwyn Bay	139	54·7	35·3	6·5	3·6	1·8	*1·96*	74·6	21·1	4·3
Ruthin	118	52·5	37·3	9·3	0·8	2·8	*1·14*	71·8	22·1	6·1
Wrexham	416	47·4	38·7	12·0	1·9	3·9	*3·03*	68·1	23·7	8·2
Ruabon	108	48·1	39·8	10·2	1·9	3·4	*0·54*	68·9	24·2	6·9
Corwen	128	56·3	29·7	11·7	2·3	2·6	*0·03*	74·9	17·7	7·4
Dolgelley	149	51·0	35·6	10·1	3·4	2·9	*0·07*	71·6	21·7	6·7
Llanfyllin	105	53·3	31·4	9·5	5·7	2·4	*3·70*	74·3	19·4	6·4
Llanfair Caereinion	107	54·2	33·6	9·3	2·8	2·5	*0·04*	73·8	20·1	6·1
Oswestry	397	52·9	36·8	8·1	2·3	2·4	*0·01*	72·7	22·0	5·3
Welshpool	378	54·5	32·5	10·1	2·9	2·5	*0·15*	74·0	19·5	6·5
Newtown	273	48·0	43·2	5·9	2·9	2·2	*0·54*	69·5	26·3	4·1
Caersws	109	53·2	28·4	14·7	3·7	3·3	*0·04*	73·1	17·4	9·5
Llanidloes	249	50·6	32·5	12·4	4·4	3·3	*0·70*	71·5	20·2	8·3
Machynlleth	180	48·9	36·1	10·6	4·4	3·2	*0·63*	70·4	22·4	7·2
Aberystwyth	194	53·6	32·0	9·3	5·2	2·3	*4·96*	74·3	19·6	6·2
Kington	104	51·0	31·7	11·5	5·8	3·0	*1·97*	72·4	19·8	7·8
Hay	167	52·7	37·1	6·0	4·2	1·8	*4·06*	73·5	22·5	4·1
Builth	100	52·0	36·0	8·0	4·0	2·3	*0·90*	72·7	21·9	5·4
Cardigan	186	48·9	39·2	9·7	2·2	3·2	*0·43*	69·6	23·8	6·6

but little. The Upper Severn Valley must, in ancient times, have formed a vast swamp separating north from south. Place names such as Trewern (the town in the morass) or Pengwern (the end of the morass)—the latter is an old Welsh name for Shrewsbury—confirm this view. When the valley was eventually colonised, people from both north and south joined in the valleyward movement but, except in the Newtown area, the colonists from the north appear to have been numerically superior. The hill folk to the north and south who did not take part in this valley settlement retained their separate identities.

The Black Mountain, Brecknock Region. The Wye, according to Fox, was impassable even in Bronze Age times. The eventual colonisation of the valley at the foot of the Black Mountain and Epynt Mountain is thought to have occurred by a valleyward movement of mountain folk. The existence of a high *O* frequency both in the valley and on the Black Mountain seems to confirm this. No figures are available for Northern Epynt as the scattered farmsteads were acquired by the War Office and their occupants evacuated. The Herefordshire plain—the only other likely portal of entry of later migrants—does not harbour a high *O* population. In Roman times the Black Mountain and Epynt were occupied by a dark-haired people termed the Silures who regarded themselves as the aboriginal inhabitants. The name Silures has, so far, defied an explanation from Celtic sources. It would, therefore, appear that the high *O* population of the area is largely a survival from pre-Celtic times. The inhabitants are serologically more akin to the North Welsh than to their South Welsh neighbours.

The European and Mediterranean picture. Almost identical *O* frequencies with *A* and *B* frequencies very similar to those observed in North Wales are rarely found in Europe or along the Mediterranean. They occur in Ireland, in Scotland and in Iceland (no figures are available for the Isle of Man). They are not found in Cornwall—another Celtic area. Very similar frequencies have been discovered in the Ille et Vilaine and the Côtes du Nord " departements " of Brittany as well as in the Manche region. The *O* frequencies of the island of Ré bear a resemblance to those of North Wales. The Basques possess very high *O* frequencies but their *B* frequencies are the lowest recorded in Europe. Many of the Berber tribes from the Rif to the High Atlas have *ABO* frequencies very similar to those of the North Welsh. Some Tunisian Berbers and Touareg nobles have *B* gene frequencies as low as 6 per cent. whilst their *O* and *A* frequencies are hardly distinguishable from those found in North Wales. The inhabitants of Sassari in Sardinia represent an almost identical *ABO* type. (No results are available for the Balearic Isles and Corsica.) In *O* frequencies the district around Spezia and the northern hinterland of Bergamo and Brescia bear a closer resemblance to North Wales than to the remainder of Italy. In the upper Alpine valleys, among the Walser, *O* attains an exceptionally high figure. In parts of Greece, in Crete and among the Greeks of Asia Minor very high *O* frequencies have been observed. The Yürük, a nomadic people of the Taurus Mountains and the inhabitants of the Western Caucasus also display the North Welsh type of *ABO* frequencies.

With the exception of the Walser, a common factor in the distribution of peoples having *ABO* frequencies very similar to the North Welsh is their predominance on islands or on land in fairly close proximity to the sea. Archæology offers support for the theory of a human migration from the Eastern Mediterranean to Britain in

Neolithic times. It is also known that the fundamental physical type in Wales is the long-headed brunet, universally recognised as belonging to the Mediterranean race of Sergi. It has been noted that the children of certain Berber tribes, if attired in European dress, would be indistinguishable in a class of North Welsh children. There is evidence that the language spoken in Wales and Ireland, prior to the advent of Celtic, belonged to the Hamitic family. One wonders, therefore, whether the peoples having the North Welsh type of *ABO* frequencies, who are scattered from the Atlantic seaboard to the Eastern Mediterranean and the Caucasus, are all remnants of a related human stock. If so, the distribution of these frequencies would seem to offer a clue to the route taken by some of the earliest colonists of Wales. A more detailed study of this problem has been made by Mourant and Watkin (1952).

It is not possible in the present state of our knowledge to compare the *Rh* frequencies of the very high *O* population found along the Mediterranean with those of the very high *O* people of North Wales. The existing *Rh* data for Wales consist only of a mixed sample of English and Welsh elements drawn from all over the Principality but predominantly from the south. Information regarding the frequencies of the other blood group systems is even more restricted and, as a consequence, comparisons with Mediterranean observations are not possible.

(iii) *Areas of high* A *frequency*

South-East Pembrokeshire. Only one region of exceptionally high *A* gene frequency has been found in Wales, *vide* fig. 2. It lies on the south-eastern side of the Milford Haven ria in a part of the area known as "Little England beyond Wales" and extends inland as far as Narberth. Historians disagree as to the origin of the population of this region. Fleure, however, has commented upon the prevalence of Nordic types in the area. The Royal Commission on Ancient and Historical Monuments in Wales, basing its views on the prevalence of Scandinavian place names and on archæological remains, postulated the existence of a Viking settlement in this part of Pembrokeshire. Charles, on the other hand, claims that the evidence suggests no more than Norse raids. The Flemings who were transferred from the north of England to South Pembrokeshire by Henry I settled farther north-west, principally in the Hundred of Rhos. The Normans were established in strength in the area as numerous castles testify. The Irish had, for a long period, been making incursions on to the Pembrokeshire coast. The inhabitants of Devon and Cornwall or Brittany could be considered potential colonists of the area whilst an emigration from other parts of South Wales was an ever-present possibility.

The blood group findings indicate that the people of this district are different both from their Welsh-speaking neighbours to the north and east and from the remainder of Little England. They also differ

from the Dutch and Belgians who are, presumably, the modern representatives of the Flemings and from the inhabitants of Western Normandy. (No figures are available for Eastern Normandy.) Identical frequencies have not been found in Brittany but the Bigoudens

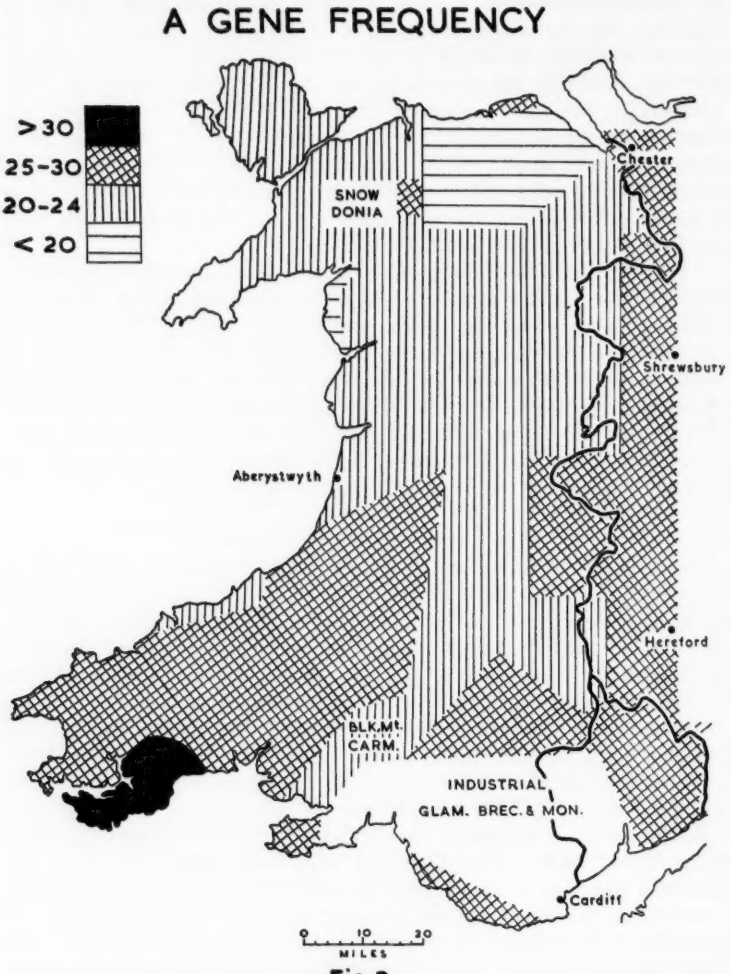

Fig. 2.

of Finisterre with an *A* gene frequency of 30 per cent. approach this South Pembrokeshire figure rather closely. Devon and Cornwall display no such high *A* frequencies whilst Ireland appears to be a nest of exceptionally low *A*. Southern Norway and the area around Stockholm, on the other hand, possess identical *ABO* frequencies. It

seems, therefore, that there was a Viking settlement in this part of Little England and that it has, to this day, maintained its genetic isolation, *vide* tables 5 and 6.

TABLE 5

High A *gene frequency areas in Wales and the Marches*

Area	Total	Group frequencies				Expected *AB* per cent.	χ^2 for homogeneity	Gene frequencies		
		O	*A*	*B*	*AB*			*O*	*A*	*B*
Little England beyond Wales (South and East of Cleddau)	431	33·9	50·1	9·7	6·3	5·3	*0·52*	58·5	33·6	7·9
Little England beyond Wales (North and West of Cleddau)	505	41·4	44·4	10·7	3·6	4·4	*0·56*	64·1	28·1	7·8
Middle Teifi Basin . . .	259	39·4	45·2	12·4	3·1	5·4	*1·61*	62·1	28·9	9·1
Mid-Cardiganshire . . .	117	40·2	46·2	9·4	4·3	4·1	*0·00(5)*	63·4	29·5	7·0
West Cheshire Plain (English surnames)	1211	41·9	46·9	8·8	2·3	3·8	*4·83*	64·3	29·3	6·4

One of the interesting features is that persons bearing Welsh surnames display a high *A* frequency, for this district is known not to have been Welsh-speaking for over eight centuries—long before the assumption of Welsh surnames. It would seem, therefore, that a substantial proportion of the inhabitants of this area assumed the Welsh type of surname despite the presumed existence of an Iron Curtain separating them from the Welsh-speaking people to the north. The contention that the majority of these people are relatively recent immigrants from Welsh North Pembrokeshire seems incorrect for no such high *A* frequency is found in the north of the county.

TABLE 6

The frequencies in the towns situated in the areas named in table 5

Town	Total	Group frequencies				Expected *AB* per cent.	χ^2 for homogeneity	Gene frequencies		
		O	*A*	*B*	*AB*			*O*	*A*	*B*
Tenby . . .	117	32·5	45·3	9·4	12·8	4·8	*9·64*	59·4	32·5	8·1
Pembroke Dock .	107	31·8	56·1	8·4	3·7	5·2	*0·27*	55·9	37·1	7·0
Haverfordwest . .	243	42·4	43·6	10·7	3·3	4·3	*0·38*	64·8	27·5	7·7
Milford Haven .	159	42·8	43·4	10·1	3·8	4·0	*0·01*	65·3	27·4	7·3
Lampeter . .	183	41·0	42·6	13·1	3·3	5·2	*0·88*	63·4	27·2	9·4
Chester (English surnames)	1091	42·0	46·9	8·9	2·2	3·9	*5·12*	64·3	29·3	6·5
Malpas (*ibid.*) . .	120	41·7	46·7	8·3	3·3	3·6	*0·02*	64·5	29·4	6·2

The Middle Teifi Basin and Mid-Cardiganshire. In the Middle Teifi Basin, from Lampeter downstream to a point beyond Llandyssul, one finds a population of higher *A* frequency than in adjoining North

Carmarthenshire. There is also an increased frequency of the *B* gene. The area, according to Fleure, is a nest of dark dolicocephals and hatters in the market town of Llandyssul are obliged to order special shapes to meet their customers' requirements. A rather similar serological type, although somewhat lower in *B*, is found in the Vale of Aeron in Mid-Cardiganshire.

Fleure, in his analysis of Cardiganshire, divided the county into three distinct regions, each with its own proportions of the various stocks. The blood group investigation confirms that North Cardiganshire differs from the middle of the county and that the south possesses characteristics of its own.

Chester and District. On the periphery of Wales lie the western portion of the Cheshire plain and the Wirral peninsula. The *A* gene frequency of 29 per cent. found among persons with English surnames at Chester contrasts markedly with the frequency of 19 per cent. found among the Welsh in the neighbouring Flintshire hills. The English town closest in latitude to Chester for which reliable figures are available appears to be Sheffield. (The calculations of Jones and Glynn for Liverpool are unfortunately based on only 40 persons.) Fisher's $A/O : A$ ratio for Sheffield $= 48{\cdot}1$ per cent. This ratio calculated for Chester $= 52{\cdot}8$ per cent.—an appreciable difference. It is to be hoped, however, that data for more English towns will be made available so that the place of Chester in the blood group map of Britain may be more accurately assessed.

The Wirral peninsula, the city of Chester and a portion of Southern Cheshire are depicted as Viking settlements in Fox's *Personality of Britain.* It is not impossible, therefore, that the increased contribution of *A* genes among the present-day inhabitants of the city is derived from a Scandinavian ancestry.

(iv) *Areas of medium* A *frequency*

General. With the exception of a part of Little England beyond Wales referred to earlier, rural South and South-West Wales have *A* frequencies ranging from 23 to 27 per cent. In the Vale of Glamorgan, the peninsula of Gower and the lower Towy valley, the *A* gene frequency exceeds 26 per cent. and approaches closely the values observed on the opposite coast of the Bristol Channel. Much of Mid-Wales also displays medium *A* frequencies. In North Wales, where very low *A* frequencies are normally found, three areas higher in *A* than the average exist. They are the island of Anglesey, the Conway Valley around Llanrwst and the Clwyd Estuary in and around Rhyl, *vide* tables 7 and 8.

South Wales. It seems that a medium *A* wave similar to that which overran Southern England penetrated most of South Wales. Intermixing with an earlier-established very low *A* population would explain the lower *A* values observed in South Wales as compared with South-Western England. Beddoe mentions that the diagram of

head-breadth indices " points towards the presence in force of at least two races in South Wales, not yet thoroughly amalgamated ". The medium *A* wave in South Wales is not of Anglo-Saxon origin for the Anglo-Saxons did not settle on the north side of the Bristol Channel. It is possible that Brittonic-speaking people whom archæologists tend to link with the Early Iron Age movement were responsible for the entry of the medium *A* frequency into South Wales.

South-Western England. Fraser Roberts' observation that Celtic Cornwall is not significantly different from the remainder of Southern England is of considerable interest. Unless the Brittonic population of Cornwall was wiped out by the Saxons, it appears that both were very similar in *ABO* blood group composition. Had the pre-Saxon

TABLE 7

The areas of medium A *frequency*

Area	Total	Group frequencies				Expected *AB* per cent.	χ^2 for homogeneity	Gene frequencies		
		O	*A*	*B*	*AB*			*O*	*A*	*B*
Anglesey	119	47·1	39·5	8·4	5·0	2·9	*1·39*	69·4	24·7	5·9
Conway Valley	135	43·7	40·7	13·3	2·2	4·9	*1·30*	65·3	25·5	9·3
Clwyd Estuary	218	43·6	42·7	9·6	4·1	3·7	*0·07*	66·2	26·9	6·9
Dyfi Basin	573	44·7	38·2	14·3	2·8	4·8	*3·36*	66·2	24·0	9·9
Teifi Basin (*a*) Lower	116	45·7	40·5	10·3	3·4	3·7	*0·01*	67·5	25·2	7·3
Teifi Basin (*b*) Upper	135	37·8	42·2	17·0	3·0	7·0	*2·03*	60·2	27·4	12·3
North Pembrokeshire	501	45·5	39·9	10·6	4·0	3·7	*0·07*	67·6	25·0	7·4
Mynydd Bach, Carmarthenshire	784	44·1	39·2	10·5	6·3	3·7	*9·58*	67·3	25·2	7·6
Lower Towy Valley and Estuary	599	47·1	42·9	7·8	2·2	2·9	*0·74*	68·4	26·2	5·5
South-Eastern Carmarthenshire	1249	48·8	37·1	11·1	3·0	3·4	*0·60*	69·7	22·7	7·5
Gower and Vale of Glamorgan	157	45·9	43·3	8·3	2·5	3·1	*0·12*	67·5	26·6	5·8
South-Eastern Monmouthshire	517	46·4	41·6	9·7	2·3	3·4	*1·37*	67·7	25·5	6·7
Archenfield	284	47·9	42·6	7·0	2·5	2·6	*0·01*	69·2	25·9	4·9

population of Cornwall been annihilated, it is unlikely that the Cornish language would have survived until the eighteenth century. That the emigrations from the Dumnonian peninsula to Brittany in the fifth to seventh centuries involved but a part of the populace seems, for the same reason, equally certain. It must, however, be remembered that even in counties such as Somerset and Dorset it is probable, according to Jackson, that the Saxon occupation was little more than a scattered settlement of Saxon masters among a subjected population. In Kent and Sussex, which lie in the zone of greatly increased frequency of Saxon place names and river names, the Saxon ethnic contribution is deemed to have been correspondingly greater. Yet Southern and South-Eastern England show no rise in *A* frequencies.

Archenfield. The " Men of Archenfield " are described as a separate entity in Domesday Book. They inhabited an area bounded by Ross, Skenfrith, Pontrilas and Aconbury. At various times in history they slew English and Welsh invaders with equal gusto. Possessing laws and customs of their own, *e.g.* " marriage and wardship are not had within the liberty of Archenfield ", these men were exempted from taxes on the condition that when the army marched into Wales they formed the vanguard and, on return, the rearguard. Eventually, most of the district was acquired as Royal property and shortly afterwards largely disafforested.

ABO blood groups suggest that Archenfield is linked with the low *B* population of the Welsh Marches rather than with South Wales.

The Dyfi Basin. The sub-division of the Dyfi Basin into two separate regions on phonological and physical anthropological grounds was undertaken by Peate. The two regions " impinge upon one

TABLE 8

The frequencies in the towns situated in table 7

Town	Total	Group frequencies				Expected *AB* per cent.	χ^2 for homogeneity	Gene frequencies		
		O	*A*	*B*	*AB*			*O*	*A*	*B*
Rhyl . . .	139	42·4	42·4	10·8	4·3	4·2	*0·00(3)*	65·2	27·0	7·8
Fishguard . .	199	43·2	39·2	11·6	6·0	4·1	*1·17*	66·4	25·3	8·3
Carmarthen . .	169	44·4	45·0	9·5	1·2	3·8	*2·03*	65·8	27·6	6·7
Llandilo . .	146	48·6	43·2	6·8	1·4	2·5	*0·51*	69·4	25·9	4·7
Ammanford . .	140	44·3	38·6	14·3	2·9	4·9	*0·81*	65·9	24·2	9·9
Burry Port . .	139	49·6	36·0	11·5	2·9	3·4	*0·09*	70·3	22·0	7·7
Llanelly . .	551	48·1	39·2	10·0	2·7	3·3	*0·40*	69·2	24·0	6·8
Abergavenny .	180	44·4	45·0	10·0	0·6	4·0	*3·57*	65·6	27·4	7·0
Monmouth . .	121	47·1	37·2	12·4	3·3	3·9	*0·09*	68·4	23·1	8·5

another along a line which runs parallel to the river on its northern side, from the direction of Aber Gynolwyn to Esgair-geiliog, and thence it follows the watershed of Ffridd Bwlch Eluan, Ffridd Cae'r Felin and Mynydd Du to the watershed between Cwm Tafolog and Cwm Nantcarfan. This boundary divides the districts of Corris-Aberllefenni and Mawddwy from those of Llanbryn Mair, Cemmaes, Machynlleth and the North Plynlymon moorland villages ", and the two districts are referred to, in brief, as " north " and " south " respectively.

Whilst the sample from the south numbers nearly 500, the one from the north is only 75. The marked excess of group *A* over group *O* in the north is in contrast to that found in the south. A larger northern sample is desirable for statistical comparison, but the available evidence suggests that Peate's differentiation of the Basin is also mirrored in the *ABO* blood group frequencies.

North Wales. Anglesey, owing to its exposed nature and its agricultural wealth,—it was for a long period the granary of all Wales—is known to have attracted the sea rovers. The Clwyd estuary faces the Wirral peninsula where Viking colonies existed. The increased number of *A* genes in these two areas may, therefore, be partly Scandinavian in origin. The recent remarkable find at Llyn Cerrig Bach, however, testifies to the activities of the Early Iron Age people in Anglesey—a people probably responsible for the introduction of the medium *A* wave into South Wales.

Llanrwst was found by Beddoe to have an index of nigrescence very considerably lower than townships in Snowdonia. Scandinavian place names are to be found at the mouth of the Conway and the river is navigable for small craft for a distance of several miles. The possibility of a sea-borne settlement at Llanrwst cannot, therefore, be excluded. On the other hand, mention has been made of a belt of lower *O* and higher *A* extending from east to west across North Wales. Corwen lies within the belt but little is known of the *ABO* blood groups in the area separating Corwen from Llanrwst. The very small samples from Pentre Foelas and Cerrig y Druidion, however, show an excess of group *A* over group *O*. It is, therefore, possible that Llanrwst is merely an extension of a bloc of raised *A* frequencies of which the Bala Cleft and the intervening area form part.

(v) *Subgroups of* A

The A_2 gene appears to be confined to populations originating in Europe, Africa and Western Asia, including India. According to Ikin, Prior, Race and Taylor, 22 per cent. of group *A* individuals from Southern England belong to subgroup A_2. From the limited data available it seems that a similar proportion of A_2 is found among Danes, Finns, Germans and Russians. In a sample of 190 from North Welsh towns, Boyd and Boyd found 35 per cent. of group *A* persons to be of subgroup A_2. Of 32 natives of the low *B* area in the Kerry Hills, whose blood was collected by the writer and tested by Mourant, 8 of the 17 group *A* persons proved to be of subgroup A_2—a high proportion. It is clearly of interest to extend the study of the *A* subgroups in Wales and in the Mediterranean peoples having *ABO* frequencies similar to the North Welsh, for the Sardinians, who have the North Welsh type of *ABO* frequencies, also possess a high proportion of A_2.

(vi) *Areas of high* B *frequency*

The *B* gene frequency is significantly higher in Western than in Eastern Wales, *vide* table 2. It reaches its highest values in isolated moorlands as may be seen from table 9. The distribution of the *B* gene frequency is depicted in fig. 3.

The elevation at which the early colonists of Britain effected their settlements increases as one proceeds southwards. In the north,

from Megalithic times until the Christian era, the high moorlands and mountains were largely uninhabited. Dartmoor, owing to its more southerly latitude, was permanently occupied even in Bronze Age times and, as a consequence, witnessed a far greater intermixing of peoples than the more northerly Welsh moorlands—a fact which may account for the absence of the more extreme frequencies of *B* and *O* found in Wales. The areas selected for habitation in Wales were the slopes between the densely-wooded valley floors and the open moors. Bronze Age finds, for example, are almost all below the 1000 ft. contour line. The distribution of hill forts which were erected from about 300 B.C. to A.D. 700 also conforms to this settlement pattern. "The old Welsh villages", as William Rees points out, "still line the hillsides at heights ranging from 500-900 ft., a fact

TABLE 9

The high B *incidence in moorland areas*

Area	Total	Group frequencies				Expected *AB* per cent.	χ^2 for homogeneity	Gene frequencies		
		O	*A*	*B*	*AB*			*O*	*A*	*B*
Ardudwy Mountain .	277	49·8	35·0	12·3	2·9	3·5	*0·23*	70·4	21·5	8·2
Mynydd Hiraethog .	181	55·2	28·7	13·8	2·2	3·0	*0·31*	74·0	17·2	8·7
Upper Dee Valley (Corwen)	128	56·3	29·7	11·7	2·3	2·6	*0·03*	74·9	17·7	7·4
Dyfi Basin . .	573	44·7	38·2	14·3	2·8	4·8	*3·36*	66·2	24·0	9·9
Plynlymon fringe (Llanidloes)	249	50·6	32·5	12·4	4·4	3·3	*0·70*	71·5	20·2	8·3
Plynlymon fringe (Caersws)	109	53·2	28·4	14·7	3·7	3·3	*0·04*	73·1	17·4	9·5
Plynlymon fringe (Rhayader)	120	40·8	44·2	11·7	3·3	4·8	*0·37*	63·4	28·1	8·5
Upper Teifi Basin .	135	37·8	42·2	17·0	3·0	7·0	*2·03*	60·2	27·4	12·3
Middle Teifi Basin .	259	39·4	45·2	12·4	3·1	5·4	*1·61*	62·1	28·9	9·1
North Brecknock and N.E. Radnor Moors	477	42·1	38·8	14·9	4·2	5·3	*0·75*	64·6	24·9	10·5
Black Mountain, Carmarthenshire	161	39·8	29·8	24·2	6·2	6·9	*0·07*	62·8	20·3	16·9

which bears out the statement of Giraldus that the Welsh were a hill people. Hence the importance in early Wales of rivers as boundaries between upland units. The occupation of the valleys would result in hill boundaries."

An invading people usually expels those whom it finds in possession of the most desirable areas of settlement and there is no reason to believe that the Bronze Age invaders of Wales were an exception to this rule. The Neolithic people who were neither killed nor enslaved were compelled to seek refuge in the high moorlands and mountains. Subsequent waves of immigrants repeated the process so that the high moors became, in time, the home of several earlier-arrived stocks. The moorland population, however, retained a nucleus derived from the very earliest arrived stock—a nucleus whose presence

Fleure was able to detect on physical anthropological grounds. In the areas mentioned by Fleure, *viz.* the Black Mountain, Carmarthenshire, the North Carmarthenshire and adjacent Cardiganshire moors and Mynydd Hiraethog, the frequencies of *O* and *A* are found to vary appreciably but a raised *B* frequency is a feature common to them all.

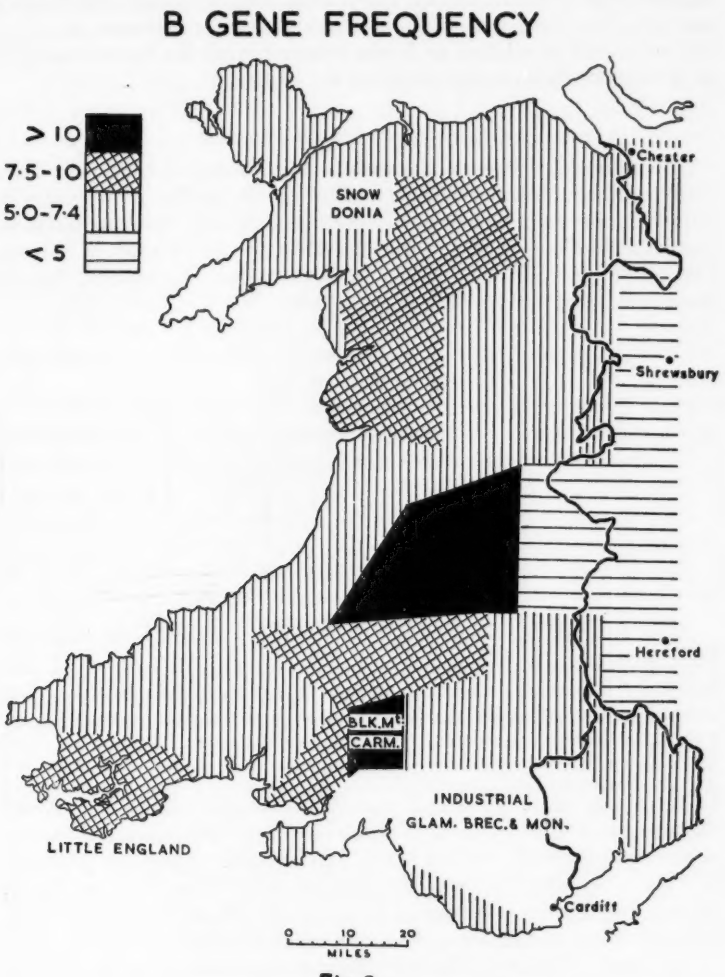

Fig. 3.

By correlating the findings of physical and blood group anthropology one is led to conclude that *B* is an ancient phenomenon in Wales and not merely a late importation from the east. The view held at present is summarised by Haldane (1940) : " Neolithic Europe was occupied

by peoples of a blood group distribution not unlike that of the American tribes, that is to say with *B* rare or absent whilst the frequencies of *O* and *A* were variable". Just as physical anthropology has shown that there was more than one variety of both Palæolithic and Neolithic Man, blood group anthropology evinces evidence that two European peoples who can lay claim to an ancient lineage, the Basques and the Moorland Folk of North Carmarthenshire, possess opposing characteristics in relation to *B*, the former having the lowest-recorded *B* in Western Europe and the latter the highest.

(vii) *An area of low* B *frequency*

A community low in *B* inhabits the Radnor Forest, the Clun Forest and the Kerry Hills, *vide* table 10. A similar *B* frequency is found among persons bearing English surnames in the neighbouring district of Ludlow, in part of the Clee Hills and in the town of Shrewsbury. The low *B* area extends southwards into Archenfield but its eastern boundary is not known.

TABLE 10

The low B *area in Wales*

Area	Total	Group frequencies				Expected *AB* per cent.	χ^2 for homogeneity	Gene frequencies		
		O	*A*	*B*	*AB*			*O*	*A*	*B*
Kerry Hills, Clun and Radnor Forests	758	45·3	46·7	5·9	2·1	2·4	*0·24*	67·2	28·6	4·3

The Basques have a very low *B* frequency and an exceptionally high proportion of *Rh* negatives. The sample of Kerry Hill folk tested by Mourant, however, showed no excess of *Rh* negatives, but the proportion of A_2, as mentioned earlier, proved to be very high.

5. SUMMARY

1. Owing to its peripheral position in relation to the Old World and the nature of its terrain, Wales is one of the few regions of Europe where traces of early human stocks still remain. Although subjected to a series of immigrations from Neolithic until Norman times, Wales escaped the full force of most of those which affected England. Even the Anglo-Saxons did not settle to the west of Offa's dyke.

2. A survey based on the *ABO* blood group results of 16,760 donors, bearing Welsh surnames, drawn from all parts of Wales other than the Glamorgan and Monmouthshire coalfield, has shown wide fluctuations in the frequencies of *O*, *A* and *B* genes. The frequencies of *circa* 3000 donors from the borderland of Cheshire, Shropshire and Herefordshire have been studied in relation to the Welsh material.

3. The *O* frequency rises as one proceeds northwards—a feature common to England. For similar latitudes, however, the *O* frequency is higher in Wales. Abrupt changes in the *O* frequency are a feature, as both sides of the Upper Severn Valley, for example, bear witness. Parts of North Wales have an *O* gene frequency of 75 per cent. whilst in a localised area in the south it falls as low as 59 per cent. There is, as expected, a marked difference between the North and South Welsh.

4. The *A* gene frequency in rural South and South-West Wales and in much of Mid-Wales ranges from 23-27 per cent. In the high *O* areas of the north it falls under 20 per cent. An extreme *A* gene frequency of 34 per cent. is found in a part of Little England beyond Wales where historians suspect a Viking settlement. A_2 appears to be unusually prevalent in Mid-Wales.

5. The *B* gene frequency is significantly higher in Western than in Eastern Wales. It reaches its peak of over 10 per cent. in isolated moorlands where physical anthropologists suggest the survival of early human stocks. This leads one to believe that *B* is an ancient phenomenon in Europe and not merely a late importation from the east.

6. The occurrence of very high *O* frequencies, together with *A* and *B* frequencies very similar to those of the North Welsh, seems confined in Western Europe and the Mediterranean to islands or to land in close proximity to the sea. From Iceland and Ireland, via islands on the French Atlantic seaboard, to the Mediterranean and white North Africa and as far east as the Aegean and Asia Minor, the North Welsh type of *ABO* frequencies is to be found. One wonders if all represent remnants of a related human stock who might be described as the " ancient mariners ".

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6. APPENDIX

(a) *Numbers observed in the high O areas (cf. table 3)*

Area	Name of town or village	Numbers			
		O	*A*	*B*	*AB*
North Caernarvonshire Coastal Plain	Llandudno	72	44	10	4
	Deganwy and Conway	23	2	1	...
	Bangor	50	49	9	2
	Port Dinorwic, Llanrug and Bethel	6	7	1	1
	Caernarvon, Waunfawr and Groeslon	93	52	14	2
	Clynnog, Llanllyfni and Llanaelhaiarn	7	6	2	2
	Totals	251	160	37	11
South Caernarvonshire Coastal Plain	Pwllheli and Abererch	49	32	15	5
	Afonwen, Chwilog and Llanystumdwy	7	7	2	...
	Criccieth	37	31	2	1
	Treflys and Borth-y-Gest	12	3	3	...
	Portmadoc	61	50	12	5
	Tremadoc, Penmorfa and Pentrefelin	9	4	3	...
	Totals	175	127	37	11
Coastal Plain of Ardudwy	Minffordd	25	13	7	4
	Penrhyndeudraeth	128	66	20	9
	Llanfrothen	18	11	2	...
	Maentwrog and Tanybwlch	17	13	5	...
	Talsarnau	51	25	16	4
	Harlech and Llanbedr	25	19	2	2
	Llanenddwyn and Dyffryn	16	6	1	...
	Talybont and Llanaber	7	6	3	...
	Barmouth	20	5	3	1
	Totals	307	164	59	20
Ardudwy Mountain	Croesor and Tanygrisiau	16	5	2	1
	Blaenau Ffestiniog	31	17	7	3
	Bethania, Manod and Ffestiniog	10	16	3	...
	Trawsfynydd and Cwm Prysor	81	59	22	4
	Totals	138	97	34	8
Denbighshire Coastal Plain, Clwyd Valley and Eastern Flintshire	Colwyn Bay and Old Colwyn	76	49	9	5
	Abergele	34	24	7	2
	Rhuddlan and Dyserth	11	6	3	...
	Bodelwyddan	12	3	2	...
	St Asaph, Tremeirchion and Trefnant	22	12	4	...
	Denbigh	16	12	2	2
	Llandyrnog and Rhewl	11	8	1	...
	Ruthin	62	44	11	1
	Llanbedr and Llanfair D.C.	11	3	2	...
	Mostyn, Holywell, Halkyn and Flint	71	30	11	1
	Northhop and Mold	46	24	9	1
	Connah's Quay, Shotton, Hawarden and Buckley	81	47	6	3
	Totals	453	262	67	15

Area	Name of town or village	Numbers			
		O	*A*	*B*	*AB*
Ruabon-Ruthin Moorlands and Middle Dee Valley	Llanferres, Llanarmon and Llandegla	10	10	1	...
	Coedpoeth	25	18	8	2
	Wrexham	197	161	50	8
	Marchwiel	11	4	1	2
	Rhostyllen	12	3	...	1
	Rhosllanerchrugog	10	6	...	...
	Penycae	8	10	3	...
	Ruabon and Rhosymedre	52	43	11	2
	Acrefair	14	11	2	2
	Cefn Mawr	19	14	...	...
	Froncysyllte	13	12	2	1
	Trevor	12	5	1	5
	Llangollen	46	30	8	2
	Glyndyfyrdwy and Carrog	13	12	3	1
	Totals	442	339	90	26
Mynydd Hiraethog	Llanefydd and Llanfair Talhaiarn	8	5	...	1
	Llangerniew and Pandy Tudor	18	5	5	...
	Gwytherin and Nebo	17	8	4	...
	Llansannan, Bylchau and Nantglyn	13	6	2	...
	Pentre Foelas and Cerrig y Druidion	7	9	1	...
	Cyffylliog	8	5	1	...
	Clawddnewydd	10	4	5	2
	Gwyddelwern	11	4	3	1
	Bettws Gwerfil Goch	8	6	4	...
	Totals	100	52	25	4
Bala Cleft and its Branches	Corwen	72	38	15	3
	Cynwyd	13	7	2	...
	Llandrillo	11	10	2	...
	Llandderfel	6	8	2	...
	Llanfor and Bala	38	26	9	6
	Llangower and Llanuwchllyn	7	7	2	...
	Rhydymain and Bontnewydd	10	3	10	3
	Brithdir	7	7	2	1
	Dolgelley and Llanelltyd	76	53	15	5
	Bontddu and Arthog	12	10	4	1
	Abergynolwyn and Bryn Crug	8	4	...	1
	Llanegryn	8	6	...	1
	Towyn	13	6	1	1
	Totals	281	185	64	22
North Montgomeryshire and fringes of adjacent Denbighshire and Shropshire	Chirk	36	23	4	3
	Llansantffraid D.C. and Tregeiriog	30	30	7	4
	Llanarmon D.C.	13	6	4	...
	Selattyn, Rhyd y Croesau and Llansilin	7	6	2	1
	Trefonen	9	4	...	...
	Llangedwyn and Pentre'r Felin	7	6	...	...
	Llanrhaiadr ym Mochnant	9	5	3	1
	Penybont Fawr	5	8	...	...
	Llangynog	10	3	1	...
	Llanwddyn	14	9	1	1
	Llanfyllin	56	33	10	6
	Meifod	18	11	2	2
	Llanfihangel yng Ngwynfa and Pontrobert	7	9	2	...

Area	Name of town or village	Numbers			
		O	*A*	*B*	*AB*
North Montgomeryshire —*contd.*	Dolanog	11	6	...	1
	Llanerfyl and Foel	7	7	2	...
	Llanfair Caereinion and Melin-y-ddol	58	36	10	3
	Castle Caereinion and Cyfronydd	8	6	1	...
	Llanwyddelan and Tregynon	22	13	2	2
	Bettws Cedewain	7	6	...	...
	Totals	334	227	51	24
Upper Severn System	Oswestry	210	146	32	9
	Llanymynech	25	12	2	2
	Llansantffraid ym Mechain	27	33	4	...
	Llanfechain	10	5	2	...
	Four Crosses and Llandysilio	11	6	1	1
	Llandrinio and Criggion	22	11	5	1
	Sarnau and Arddleen	8	5	...	1
	Pool Quay	8	12	...	...
	Guilsfield	8	7	...	...
	Buttington and Trewern	14	9	3	1
	Welshpool	206	123	38	11
	Leighton	8	11	6	1
	Forden	12	15	1	1
	Chirbury	30	7	5	2
	Churchstoke	36	22	4	2
	Berriew and Garthmyl	19	26	9	3
	Abermule and Llanmerewig	13	9	1	1
	Llanllwchaiarn and Aberbechan	5	10	...	...
	Newtown	131	118	16	8
	Aberhafesp	11	10	1	...
	Caersws	58	31	16	4
	Llanwnog, Pontdolgoch and Clatter	18	6	2	2
	Llandinam	53	28	7	...
	Llanidloes	126	81	31	11
	Trefeglwys	13	9	1	1
	Carno and Talerddig	38	27	5	4
	Totals	1120	779	192	66
Dyfi Basin	Llanbrynmair	20	27	5	3
	Aberhosan, Dylife and Pennant	4	9	5	...
	Comins Coch, Tafolwern and Talywern	6	12	...	...
	Cemmaes and Abercegir	27	13	5	...
	Cwmllinau	8	12	4	1
	Aberangell, Mallwyd and Dinas Mawddwy	9	10	3	...
	Llanwrin	14	5	2	...
	Penegoes and Forge	14	9	4	...
	Pantperthog and Ceinws	5	10	3	1
	Corris and Aberllefenni	11	17	4	2
	Machynlleth	88	65	19	8
	Pennal and Derwenlas	21	5	13	1
	Ynyslas and Llancynfelin	17	14	9	...
	Aberdyfi	12	11	6	...
	Totals	256	219	82	16
Aberystwyth-Plynlymon Area	Van (Llanidloes)	7	9	1	2
	Llawr-y-Glyn	5	9	2	...
	Staylittle	12	2	...	...

Area	Name of town or village	Numbers			
		O	*A*	*B*	*AB*
Aberystwyth-Plynlymon Area—*contd.*	Cwmbellan and Llangurig	13	6	2	1
	Ponterwyd and Goginan	17	8	1	...
	Capel Bangor	18	7	5	1
	Penrhyncoch, Penybont Rhyd-y-Beddau and Cwmsymlog	15	12	5	2
	Pontgoch and Talybont	10	16	2	1
	Taliesin and Tre'rddol	11	10	1	...
	Penygarn and Bow Street	33	26	3	...
	Aberystwyth	104	62	18	10
	Llanrhystyd and Llanon	11	5	2	...
	Totals	256	172	42	17
Coastal South Cardiganshire	New Quay	21	13	4	3
	Llangranog, Tresaith and Aberporth	7	6	...	2
	Blaenporth and Beulah	7	6	1	1
	Cardigan	91	73	18	4
	St Dogmaels	10	3	2	1
	Totals	136	101	25	11
Upper Wye, Epynt, Black Mountains of Brecknock and Upper Usk Area	Builth	52	36	8	4
	Aberedw and Erwood	26	17	3	1
	Llanstephan	6	7	3	...
	Hay	88	62	10	7
	Kington	53	33	12	6
	Lyonshall	18	18	4	1
	Eardisley	14	13	1	1
	Whitney and Willersley	15	8	3	1
	Bredwardine	9	5	1	...
	Tyberton and Madle	11	9	2	...
	Kingstone	9	8	1	...
	Upper Chapel	9	4	...	...
	Llangorse and Bwlch	6	8	4	...
	Cwmdu	8	5	...	...
	Llanbedr, Patrishow and Cwmyoy	8	6	...	1
	Capel-y-Ffin, Llanthony and Longtown	11	3	2	...
	Dorstone	9	12	2	...
	Peterchurch	10	4	2	...
	Vowchurch	14	7	...	1
	Abbeydore and Ewyas Harold	12	8	3	...
	Pontrilas	9	10	4	1
	Pandy and Llanfihangel Crucorney	6	10	4	...
	Gilwern and Llangrwyney	16	13	3	1
	Llangenau	10	7	3	...
	Crickhowell	31	28	7	4
	Llangattock	20	10	...	1
	Ffawyddog	9	4	1	...
	Llangynidr	12	7	2	...
	Brecon	34	27	9	1
	Totals	535	389	94	31

(b) *Numbers observed in the high* A *areas (cf. table* 5)

Area	Name of town or village	Numbers			
		O	*A*	*B*	*AB*
Little England beyond Wales (North and East of Cleddau)	Llawhaden and Robeston Wathen	5	8	3	...
	Narberth and Templeton	28	46	7	2
	Cold Blow, Ludchurch, Tavernspite	5	8	3	1
	Tenby	38	53	11	15
	Cosheston, Milton and Carew	5	8	1	1
	Lamphey, Manorbier, St Florence and Penally	9	7	2	...
	Pembroke Dock	34	60	9	4
	Pembroke, Monkton, Castlemartin and Stackpole	22	26	6	4
	Totals	146	216	42	27
Little England beyond Wales (South and West of Cleddau)	Roch, Camrose, Prendergast and Withybush	6	10	2	1
	Haverfordwest	103	106	26	8
	Merlin's Bridge, Lower Freystop, Tiers Cross, Johnston and Llangwm	6	7	1	1
	Llanstadwell and Neyland	7	10	4	...
	Milford Haven	68	69	16	6
	Hakin	14	12	4	2
	Talbenny, Hasguard, Marloes and Dale	5	10	1	...
	Totals	209	224	54	18
West Cheshire Plain	Chester (English Surnames)	458	512	97	24
	Malpas (English Surnames)	50	56	10	4
	Totals	508	568	107	28
Mid-Cardiganshire	Aberaeron	30	30	6	4
	Ciliau Aeron, Ystrad, Talsarn and Llangeitho	8	6	2	...
	Llanarth, Mydroilyn and Dihewid	7	7	2	1
	Cribyn and Cwrtnewydd	2	11	1	...
	Totals	47	54	11	5
Teifi Basin (*b*) Middle	Velindre, Llangeler and Pentrecwrt	8	11	3	...
	Pont Tyweli and Llandyssul	5	8	2	2
	Llanfihangel ar Arth, Maes y Crugiau and Llanwenog	6	7	1	...
	Llanybyther, Pencarreg and Llanwnen	8	13	2	...
	Lampeter	75	78	24	6
	Totals	102	117	32	8

(c) *Numbers observed in the medium* A *areas (cf. table* 7)

Anglesey	Holyhead	48	40	7	3
	Llangefni, Llanfair P.G. and Beaumaris	8	7	3	3
	Totals	56	47	10	6
Conway Valley	Llanrwst . . . . . Total	59	55	18	3

Area	Name of town or village	Numbers			
		O	*A*	*B*	*AB*
Clwyd Estuary	Towyn on Sea	9	7	2	1
	Rhyl	59	59	15	6
	Prestatyn	27	27	4	2
	Totals	95	93	21	9
Teifi Basin (*a*) Lower	Llangoedmor and Cilgerran	8	10	2	...
	Llechryd, Llandygwydd, Abercych, Cenarth and Cwmcoy	11	8	4	1
	Newcastle Emlyn	28	21	6	1
	Adpar, Llandyfriog and Henllan	6	8	...	2
	Totals	53	47	12	4
Teifi Basin (*c*) Upper	Silian, Derry Ormond and Cellan	6	7	3	...
	Llangybi and Llanfair Clydogau	3	10	1	...
	Pont Llanio and Llanddewi Brefi	13	4	5	1
	Tregaron	24	28	12	2
	Pontrhydfendigaid, Ystrad Meurig and Ffair Rhos	5	8	2	1
	Totals	51	57	23	4
North Pembrokeshire	Llantood, Newport, Nevern and Eglwyswrw	17	15	5	...
	Boncath and Blaenffos	12	3	1	...
	Crymmych, Hermon, Llanfyrnach and Pentre Galar	9	4	1	...
	Dinas Cross and Llanychaer	6	8	1	...
	Rosebush, Mynachlogddu and Maenclochog	9	5	...	...
	Llandissilio and Clynderwen	11	4	...	...
	Fishguard	86	78	23	12
	Llanwnda and Goodwick	27	27	5	...
	Letterston	19	27	5	2
	Little Newcastle and Puncheston	9	6	2	...
	Wolf's Castle	9	9	4	3
	Ambleston and Trefgarn	6	8	4	2
	Mathry, Groesgoch, St David's and Solva	8	6	2	1
	Totals	228	200	53	20
North Carmarthenshire	Trelech	10	9	3	3
	Talog, Blaenycoed and Conwil Elvet	15	8	4	5
	Pencader	3	15	2	1
	Llanpumpsaint, Alltwalis and Rhydargaeau	12	8	3	1
	Llanfihangel Rhos y Corn, Gwernogle, Brechfa and Abergorlech	7	7	...	...
	Llanfynydd and Capel Isaac	12	15	1	1
	Salem	20	7	3	...
	Taliaris and Talley	32	23	5	6
	Llansawel	20	22	5	3
	Crugybar	12	14	6	1
	Pumpsaint	33	22	11	6
	Ffaldybrenin and Farmers	14	14	1	1
	Caio	12	21	10	1
	Rhandirmwyn	19	17	1	1
	Cilycwm	12	12	4	3
	Cynhordy	16	11	2	4

Area	Name of town or village	Numbers			
		O	*A*	*B*	*AB*
North Carmarthenshire —*contd.*	Llandovery	34	27	8	4
	Llanwrda	14	10	3	3
	Llansadwrn	13	6	4	2
	Llangadog	36	39	6	3
	Totals	346	307	82	49
Lower Towy Valley and Estuary	Llandilo and Ffairfach	71	63	10	2
	Broadoak ond Llangathen	9	5	2	...
	Golden Grove	8	6	1	...
	Dryslwyn	24	9	2	2
	Llanarthney, Llanegwad and Pont ar Gothi	7	6	3	1
	Nantgaredig, Felinwen, Abergwili and Llangunnor	24	24	3	2
	Carmarthen	75	76	16	2
	Llanllwch, Llangain and Llanstephan	9	7	1	1
	Ferryside	9	11	1	...
	Llanybri, Llangynog and Bancyfelin	12	7	2	1
	St Clears and Llangynin	15	21	3	1
	Laugharne	4	9	2	...
	Whitland	15	13	1	1
	Totals	282	257	47	13
South-Eastern Carmarthenshire	Cwmllynfell	45	20	8	2
	Brynamman, Garnant and Glanamman	16	17	4	...
	Llandybie	24	15	5	...
	Ammanford	62	54	20	4
	Penybank	19	9	5	2
	Saron	13	17	4	2
	Capel Hendre	12	9	2	...
	Penygroes, Gorslas and Cross Hands	12	7	2	...
	Tumble	15	17	5	3
	Trimsaran	20	10	4	3
	Burry Port	69	50	16	4
	Llanelly	265	216	55	15
	Felinfoel and Llwynhendy	29	15	5	1
	Llangennech	9	7	4	1
	Totals	610	463	139	37
Gower and Vale of Glamorgan	Llanmadoc, Llangennith and Rhossily	12	15	5	1
	Llanrhidian, Llanmorlais and Penclawdd	15	7	1	2
	Penard and Bishopston	6	8	1	...
	Llangan, Colwinston and Llysworney	6	7	...	...
	Aberthin, Cowbridge and Llanblethian	9	5	2	1
	Llantwit Major, Boverton and Flemingston	8	11	...	...
	Gileston and Penmark	8	5	1	...
	Llanilltern, Bonvilston and Llancarfan	8	10	3	...
	Totals	72	68	13	4
Eastern Monmouthshire	Abergavenny	80	81	18	1
	Llanvetherine and Cross Ash	10	4	1	...
	Llandeilo Croeseny, Llanfihangel Y.L. and Penrhos	7	7	1	...

Area	Name of town or village	Numbers			
		O	*A*	*B*	*AB*
Eastern Monmouthshire —*contd.*	Llangattock V.A., St Maughan's Rockfield and Hendre	9	7	...	1
	Llanfoist, Llanelen, Llanover and Usk	13	5	1	1
	Raglan, Dingestow and Mitchel Troy	5	8	2	1
	Monmouth	57	45	15	4
	Dixton, Penallt and the Kymin	6	7	1	...
	Redbrook, Whitebrook and Llandogo	6	17	2	1
	Trelleck	11	13	3	1
	Llanishen and Devauden	7	6	3	...
	Tintern	13	1	2	...
	St Arvan's	7	6	...	...
	Chepstow	9	8	1	2
	Totals	240	215	50	12
Archenfield	Garway, Orcop and Much Birch	22	17	...	2
	Skenfrith and Kentchurch	8	8	3	...
	Llangarren	15	12	2	...
	Llangrove	7	7	1	...
	Peterstow and Goodrich	15	11	1	1
	Broad Oak and Welsh Newtown	17	11	2	2
	Hoarwithy	8	7	...	...
	Little Dewchurch	15	13	4	1
	Ross	29	35	7	1
	Totals	136	121	20	7

(d) *Numbers observed in the high* B *areas* (*cf. table* 9)

North Brecknock and North Radnor Moors	Llanwrtyd, Llanddulas and Llangammarch	35	27	11	3
	Abergwessin and Beulah	10	5	5	1
	Newbridge and Llanyre	12	12	...	...
	Llandrindod and Llanbadarn Fawr	20	18	11	2
	Penybont, Llandegley and Llanbister	5	10	1	3
	Nantmel and Llanwrthwl	12	6	11	...
	Rhayader	49	53	14	4
	Cwmdauddwr and Elan Valley	5	13	2	...
	St Harmon	6	7	7	...
	Pantydwr and Tylwch	14	5	4	3
	Abbeycwmhir	13	13	1	...
	Dolfor, Mochdre, Llanbadarn Fynydd	20	16	4	4
	Totals	201	185	71	20
Black Mountain, Carmarthenshire	Bethlehem	27	17	15	3
	Gwynfe	12	6	7	1
	Llanddeusant	9	11	5	3
	Myddfai	12	4	4	1
	Cwmwysg	4	10	8	2
	Totals	64	48	39	10

(e) *Numbers observed in the low* B *area (cf. table* 10)

Area	Name of town or village	Numbers			
		O	*A*	*B*	*AB*
Radnor Forest, Clun Forest, Cefn Coed and Kerry Hills	New Radnor, Kinnerton and Evanjobb	7	7	...	...
	Titley and Kinsham	31	31	5	2
	Presteign	28	25	1	1
	Lingen	6	8	...	...
	Stapleton, Norton, Whitton, Discoyd and Cascob	7	6	...	...
	Knighton	23	28	4	1
	Knucklas, Skyborry, Gwernafal and Rhos-y-meirch	6	10	...	...
	Llanfair Waterdine, Beguildy and Bettws-y-crwyn	4	15	...	...
	Bucknell	48	33	5	3
	Leintwardine	12	9	1	1
	Clun, Clunton, Clunbury, Aston on Clun, and Clungunford	44	34	8	1
	Lydbury North	3	11	1	...
	Bishop's Castle	38	42	5	2
	Montgomery	42	45	9	2
	Hyssington and Lydham	7	6	...	1
	Sarn and Llandyssil	5	19	2	...
	Kerry	20	14	4	...
	Cefn Coed	12	11	...	2
	Totals	343	354	45	16

* Grand Total for Wales	16,760	8124	6401	1681	554
Total for North Wales	4535	2351	1581	462	135
Total for Mid-Wales	5088	2510	1936	483	159
Total for South Wales	7137	3257	2884	736	260
Total for Western Wales	8632	4053	3317	942	320
Total for Eastern Wales	8128	4071	3084	739	234

* *N.B.*—The figures for Chester and Malpas are not included in the Welsh totals.

(f) *List of Welsh surnames* †

Adda	Dilwyn	Gwyn	Kyffin	Phillips	Rogers
Anwyl	Edwards	Gwyther	Lewis	Powell	Rowlands
Bevan	Elias	Harries	Leyshon	Powis	Thomas
Beddoe	Evans	Hopkin	Llewellyn	Preece	Treharne
Bellis	Eynon	Howell	Lloyd	Price	Trevethan
Bithel	Francis	Hughes	Loughor	Pritchard	Trevor
Blythin	Gethin	Humphreys	Machen	Probert	Tudor
Bowen	Glyn	Idris	Maddock	Probyn	Vaughan
Breese	Gough	Ithell	Meredith	Prothero	Walters
Cadarn	Griffith	James	Meyrick	Prytherch	Watkin
Caddell	Gronow	Jenkins	Morgan	Pugh	Welsh
Cadwallader	Gwatkin	John	Morris	Rees	Williams
Craddock	Gwilt	Jones	Owen	Richards	Wynn
Davies	Gwilym	Kenwyn	Parry	Roberts	Yorath

† Many names have variant spellings which may also be considered Welsh.

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GENETICS OF FRAGMENT CHROMOSOMES IN *TRILLIUM GRANDIFLORUM*

ALFRED RUTISHAUSER
Institut für allgemeine Botanik, Universität Zürich

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1. SUPERNUMERARY CHROMOSOMES

A CONSIDERABLE number of organisms exhibit, as well as the normal chromosomes of the diploid chromosome set, B-chromosomes which behave in an irregular manner partly from a morphological point of view, but more often from the physiological. Although it is usually easy to distinguish between the B- and the ordinary or A-chromosomes, there are many difficulties in the way of finding features which are characteristic of all known B-chromosomes.

Oestergren (1945) tried to find a universally valid definition by grouping together all known characteristics of B-chromosomes. But he himself points out that no single B-chromosome possesses all the characteristics mentioned.

B-chromosomes clearly represent an extraordinary divergent group of chromosome bodies, and at present it is not easy to see how, for example, the big gap between the accessory chromosomes of *Godetia viminea* (Håkansson, 1949, 1950) and the B-chromosomes of *Zea Mays* (Randolph, 1941) can be bridged. Further extensive research is necessary to clarify the origin and function of these interesting chromosomes.

The present work is a report on several experiments which were made with the particular object of investigating the distribution of fragment chromosomes of *Trillium grandiflorum*. The results obtained throw some light on their distribution during the heterotypic division of the EMC. Because of the great difficulties of breeding from *Trillium*, the investigations into fragment chromosomes were undertaken with hybrid endosperms. Because one of the ordinary chromosomes of *T. grandiflorum* is termed a " B-chromosome ", we are forced to use the expression " fragment " for the supernumerary chromosomes.

2. MATERIAL AND METHODS

The test-plants, 75 specimens of *T. grandiflorum* and 3 of *T. luteum* were taken from three nurseries—A. Vogt, Erlenbach ; C. Frickart Söhne, Stäfa/Zürich (both classified G) and B. Ruys, Dedemsvaart/Holland (GH). The plants from all three sources proved to be highly self-sterile.

To obtain information about the heterochromatin content, all plants were exposed to a temperature of 0·3° to 1·0° C. for four days. This was before fixation, five or six weeks after pollination. My experiments show that this treatment is sufficient to obtain maximum starvation effect with normal chromosomes of root tip and endosperm mitosis.

After the exposure to cold the seeds are taken out of the fruit, fixed in Carnoy and then transferred to 80 or 90 per cent. alcohol. In this fluid, the seeds can be

kept up to three weeks at a temperature of 0° C. without the chromosomes losing their staining capacity. After the hydrolysis (six to ten minutes in *n*HCl at 60° C.) and staining in leuco-basic fuchsin the endosperms can easily be extracted from the seeds with the help of two needles. As Feulgen-stain often penetrates slowly, it is recommended that the seeds be taken out of the staining solution after two hours and left in tap water for two or three hours. Staining with Orcein has also proved useful.

3. RESULTS OF THE EXPERIMENTS

As is shown in table 1, plants with fragments appear in both samples of *T. grandiflorum* ($2n = 10$). Of 54 plants from the G-sample, 10 specimens contained one and two contained two fragments.

TABLE 1

Fragment chromosomes in T. grandiflorum *and* T. luteum

Plant	*n*	No. of standard fragments			Telocentrics
		0	1	2	
T. grandiflorum G . .	54	42	10	2	...
T. grandiflorum GH .	21	17	1	...	3
T. luteum . . .	3	2	1	...	...

All the fragments in this first experimental group were morphologically identical apart from minor differences of size. They possess a submedian centromere, the shorter arm measures 0·9 μ on the average, the longer one 1·3 μ (text-fig. 1*a* ; plate, fig. 1). No signs of heterochromatin could be discovered. The staining even after prolonged cold treatment (4-5 days in $\pm$0° C.) coincides completely with the staining of the euchromatic regions of the ordinary chromosomes. Additional heteropycnotic spots in the resting nucleus could not be discovered. The fragments of the G-plants are, therefore, completely euchromatic. We term them hereafter standard fragments (*fr*).

TEXT-FIG. 1.—Fragment chromosomes ; *a*, *b*, standard fragments from *T. grandiflorum* (*a*, G, *b*, GH) ; *c*, telocentric ; *d*, standard fragment from *T. luteum*. ×5500.

The fragments of the second experimental group were also purely euchromatic. But here two different kinds of *fr*-chromosomes appeared. One kind (text-fig. 1*b*) coincides with the standard fragment of the

G-group. The centromere of the other kind, however, seems to be terminal (text-fig. 1*c*; plate, fig. 2) even in the best preparations. No trace of a second shorter chromosome arm could be found. Numerous measurements have shown that their centromere region is of the same length as that of the standard fragments. I am, therefore, of the opinion that here it is a question of genuine telocentric chromosomes (*t*). The length of its arm is 3·5 μ. It is considerably bigger than the sum of the arms of the standard fragment. Therefore, it does not seem probable that they have resulted from a break at the centromere followed by reunion of the shorter and longer arms of the standard fragment.

The distribution of both types of chromosomes, the standard fragment and the telocentric chromosome, in the mitosis of the root tips and the endosperms is completely normal. No somatic non-disjunction could be found.

The *fr*-chromosomes of *T. luteum* ($2n = 10$) correspond morphologically to the standard type of *T. grandiflorum* (text-fig. 1*d*). They too are purely euchromatic and possess a submedian centromere. The length of the two arms is on an average smaller. They are 0·5 μ and 1·0 μ respectively.

4. DISTRIBUTION OF FRAGMENT CHROMOSOMES IN THE ENDOSPERMS

The distribution of the *fr*-chromosomes could only be exactly ascertained in the standard fragment of *T. grandiflorum*. In 18 experimental plants crossbreedings of the type O×*fr*, *fr*×O and *fr*×*fr* was

TABLE 2

Distribution of fragment chromosomes in F_1-endosperms

Cross	No. of crosses	No. of fragments in endosperms				*n*	*fr* per endosperm
		0	1	2	3		
1. *Standard fragments*							
O×*fr* . .	*16*	166	183	...	...	349	0·5
fr×O . .	*6*	27	2	175	1	205	1·7
fr×*fr* . .	*4*	5	3	33	21	62	2·1
2. *Telocentrics*							
t×O . . .	*1*	2	...	2	...	4	...

carried out, and the number of *fr*-chromosomes in F_1-endosperms was investigated with squash preparations (plate, figs. 3-5). The results of these investigations are reproduced in table 2.

The cross O×*fr* gave a normal 1 : 1 distribution. The deviation is not significant ($P \geqslant 0{\cdot}5$). In agreement with Sparrow's statements (1952) about the behaviour of the *fr*-chromosomes in the meiosis of

T. erectum it can be stated that the distribution of the fragments in the microspores is regular. During pollen grain-development non-disjunction does not occur or is very rare.

On the other hand, the distribution of the standard fragments on the female side deviates noticeably from the ratio 1 : 1. As the endosperm nuclei are made up of two polar nuclei and one sperm nucleus, we would expect endosperms without standard fragments and those with two standard fragments in equal numbers. Instead of this, there was a considerable predominance of 2 *fr*-endosperms. As the χ^2-test shows, the deviation from the 1 : 1 ratio is significant ($P<0{\cdot}001$).

That this distribution is not accidental is further proved by the results of crosses carried out on plants which contained a *fr*-chromosome each. In the *fr* × *fr*-crosses, the endosperms with and without a *fr*-chromosome correspond to the O-endosperms of the *fr* × O-cross, the endosperms with 2 or 3 *fr*-chromosomes respectively correspond with the 2 *fr*-endosperms. The frequency of the last two categories is 87·1 per cent. and is almost exactly the same as the frequency of the two *fr*-endosperms in the *fr* × O-cross (85·9 per cent.). Therefore, there can be no doubt that distribution of the standard fragments in male and female gametes does not take place according to the same laws.

The question arises how the uneven distribution of standard fragments in the ovules occurs. Although no investigations into the embryo sac-development of *Trillium* plants with such chromosomes have been carried out, their behaviour during meiosis can be inferred. First it must be remembered that the embryo sacs of *T. grandiflorum* develop according to the *Allium*-type (Ernst, 1902). After the heterotypic division of the EMC 2 dyad cells appear. One of these, the micropylar, degenerates, while the chalazal dyad cell without further division develops into the nuclear embryo sac.

From the development of the embryo sac of *T. grandiflorum* it can be seen that the deviation from the ratio 1 : 1, as found in the distribution of standard fragments in the endosperms of *fr* × O- and *fr* × *fr*-crosses, can only be due to the fact that the univalent *fr*-chromosomes are irregularly distributed during the first meiotic division. According to Sparrow (1952), the unpaired *fr*-chromosomes of *T. erectum* remain undivided in the first division. They do not split until the anaphase of the second division. Assuming that the univalents behave in the same way in the meiosis of the EMC of *T. grandiflorum*, the conclusion can be drawn that their movement during the first division is directed. More standard fragments move to the chalazal than to the micropylar pole. By regular distribution of the split halves into the two nuclei of the two-nuclear embryo sac (second division) only nuclei with fragments arise in the female gametes. So it can be understood why two standard fragments were found in most endosperms.

The preponderance of dyads with standard fragments would also be comprehensible if we assume that their split takes place at least

in part in the second division as Bosemark (1954) states in connection with *Festuca pratensis* and Fernandes (1946) with *Narcissus bulbocodium*. But as mostly endosperms with two standard fragments appear, it would further have to be assumed that all fragment chromosomes split in the second meiotic division again. Such behaviour is assumed by Fernandes (1946) for single B-chromosomes of *Narcissus bulbocodium*. Apart from the fact that this conclusion was arrived at only indirectly (from the distribution of heterochromatin in the resting nucleus of the microspores), the assumption of an occasional additional division of univalents in the second meiotic division is not sufficient to explain the frequent appearance of endosperms with two standard fragments. Therefore, I keep to my first assumption.

Unfortunately, the genetics of telocentrics could not be investigated thoroughly. All three experimental plants with such chromosomes were weakly and only one flowered. In the one fruit obtained only six seeds developed. In four endosperms of the *fr* × O-cross the number of telocentrics could be ascertained. Two were free of them, and two had two telocentrics each. Plate, fig. 6 shows a metaphase

TABLE 3

Seed fertility of T. grandiflorum

Cross	No. of fruit	Well-developed seeds	Degenerate seeds	Seed fertility	*fr* per endosperm
O × O . .	12	220	168	56·7 per cent.	0·0
O × *fr* . .	20	600	202	74·8 ,,	0·5
fr × O . .	6	198	90	68·8 ,,	1·7
fr × *fr* . .	4	82	23	78·1 ,,	2·1

with $6n = 30$ chromosomes which was caused by the duplication of the triploid chromosome number $3n = 15$ (a process which occurs relatively often in endosperms) and in which four telocentrics can clearly be seen.

While the telocentrics seem to have a detrimental influence, the standard fragments in the quantity observed appear to have a favourable effect on the seed fertility.

Table 3 summarises the results of counts which were made of the seeds of six- to seven-weeks-old fruits of *T. grandiflorum*. They show that there is a slight increase in the number of well developed seeds in fruits of the crosses O × *fr*, *fr* × O and *fr* × *fr*. But it must be stated that the ordinary chromosomes of several plants which are used in these crosses vary in their heterochromatin content. The heterochromatin seems to have some influence on the seed fertility. This and the most striking effect of the *fr*-chromosomes of *T. grandiflorum*, their ability of promoting spontaneous chromosome breakage, will be discussed in another paper.

5. DISCUSSION

In *Trillium* fragment chromosomes were first discovered by Sparrow, Pond and Sparrow (1952) in *T. erectum*, and their behaviour during meiosis and pollen development was investigated. According to Sparrow's statements, they can be regarded generally as fairly normal. In the first meiotic division they move undivided to one of the two poles and split in the second division. Loss of fragment chromosomes through lagging occurs, but is relatively rare. So in plants with only one fragment chromosome, the values found for microspores with and without a fragment deviate slightly from the ratio 1 : 1. Microspores without fragments are somewhat more frequent.

The results of the cross experiments which were carried out on *T. grandiflorum* partly agree with Sparrow's findings. The cross O $\times$ *fr* produced endosperms with and without fragment chromosomes in the ratio 1 : 1 (table 2). But, surprisingly, the reciprocal cross *fr* $\times$ O leads to quite a different result: the number of endosperms with two standard fragments is considerably higher than would be expected with random distribution (85·9 per cent. instead of 50 per cent.). The same result was given by the *fr* $\times$ *fr*-cross. As has been stated in full above (p. 198), this finding can be best understood if it is assumed that the univalent standard fragments mostly move towards those dyads which later form the embryo sac. Their movement in the first meiotic division of the EMC is, therefore, directed.

It can be stated that there is a distant similarity between the distribution described for *T. grandiflorum* and for the acrocentric B-chromosomes of *Trimerotropis sparsa* and *Circotettix thalassium*. According to White (1954), univalent B-chromosomes are distributed at random in spermatogenesis, but bivalent B-chromosomes often group themselves in such a manner on the spindle that both partners move towards the same pole. In this way, gametes are formed with two B-chromosomes and without any.

The abnormal behaviour of the standard fragments in the *fr* $\times$ O- and *fr* $\times$ *fr*-crosses indicates that the conditions to which they are subject in the PMC and EMC are somewhat different. While ordinary chromosomes (which, of course, pair regularly) are distributed evenly in both cell types, the difference is big enough to influence the distribution of the fragment chromosomes. As the standard fragments of *T. grandiflorum* are purely euchromatic, the heterochromatin is eliminated as a possible factor in the irregular course of the first meiosis. It is, therefore, very probable that the centromere has something to do with the difference. Unlike Sparrow (1952), my conclusion is that the centromere is not quite normal. Some weight is given to this finding because of Fernandes' assumption (1946) that the heterochromatisation of B-chromosomes in *Narcissus bulbocodium* resulted in a weakening of the centromere. In the standard fragment of *T. grandiflorum* no connection can be found between the chromatin type and the physiology of the centromeres.

An interesting consequence of the irregular distribution of standard fragments in the meiosis of the EMC is that there is an increase in their number in the endosperms of *fr*×O- and *fr*×*fr*-crosses. If one assumes that embryos of endosperms with one or two standard fragments all contain 1 *fr* and embryos of endosperms with 3 standard fragments contain 2, the inference is that the average fragment content in the embryos of these crosses is higher than that in the parents. For the two crosses *fr*×O and *fr*×*fr* it amounts to 0·86 and 1·26 respectively instead of 0·5 and 1·0 respectively. In spite of the failure of non-disjunction in the pollen grain mitosis, the result should, therefore, be an increase of their numbers in the F_1-generation. One may, therefore, suppose that the fragments of *T. grandiflorum* maintain or even increase their numbers in a population.

Thus, the directed distribution of standard fragments in the meiosis, like non-disjunction, shows a special mechanism which favours their propagation. It is not known whether this is counteracted by an opposing process as is the case with *Secale cereale* (Müntzing, 1943), where lagging results in a considerable loss of accessory chromosomes at meiosis and where, as the number of such chromosomes increases, the seed fertility is lowered. The *fr*×*fr*-crosses produced about the same percentage of good seeds as the O×*fr*-crosses. Fertility tests with plants with more fragment chromosomes have not yet been made.

In their tendency to maintain themselves within a population, the standard fragments of *T. grandiflorum* resemble the B-chromosomes of *Zea Mays*, *Secale cereale*, *Anthoxanthum aristatum* and *Festuca pratensis*. It is only the mechanism which produces this result that is different.

As to the chromaticity, considerable differences exist between the B-chromosomes of the plants hitherto investigated. The B-chromosomes of *Zea Mays* (Darlington and Upcott, 1941), *Anthoxanthum aristatum* (Oestergren, 1947) and *Festuca pratensis* (Bosemark, 1954) are distinctly heterochromatic. The B-chromosomes of *Sorghum purpurascens* (Darlington and Thomas, 1941), *Poa alpina* (Håkansson, 1948), *Narcissus bulbocodium* (Fernandes, 1946), *Centaurea scabiosa* (Fröst, 1948) and all the accessory chromosomes in mosses noted by Vaarama, (1949, 1950, 1953) have also been designated heterochromatic. Whether this is also true of *Secale cereale* does not seem to be certain. Müntzing (1951) is of the opinion that the accessory chromosomes of this species are not heterochromatic or, at least, not clearly so, but he adds that the standard fragments, according to the investigations of Lima de Faria, contain more regions of special appearance in proportion to their length than the A-chromosomes.

Darlington and La Cour (1938) were the first to show with fragment chromosomes of *Paris polyphylla* that the B-chromosomes can be purely euchromatic also. The same is also true for the B-chromosomes of *Caltha palustris* (Reese, 1954) and *Godetia viminea* (Håkansson, 1949).

After Sparrow (1952) had drawn attention to the euchromatic nature of the fragments of *T. erectum*, I succeeded in showing that in *T. grandiflorum* and *T. luteum*, even after prolonged cold treatment (4-5 days in a temperature of ±0° C.), there was no sign of any difference in staining between standard fragment and euchromatic ordinary chromosomes. Thus, the fragment chromosomes of the *Trillium* species, too, contain no visible heterochromatin. These findings have some significance with regard to the abnormal behaviour of the standard fragments in the course of meiosis and, especially, because of their genetic activity. It has already been shown that the standard fragments of *T. grandiflorum* tend to maintain themselves within a population as well as the heterochromatic B-chromosomes of maize and rye. But in respect of the genetic activity, too, there appears to be no essential difference, as far as present experimental results permit such a conclusion.

According to my investigations the seed fertility seems to be favourably influenced by the introduction of up to three euchromatic standard fragments in the endosperm. Favourable effects are described for heterochromatic B-chromosomes as well. From Fröst's measurements of plant weight of *Centaurea scabiosa* (cit. from Müntzing, 1954) the presence of a few B-chromosomes seems to be more advantageous than their complete absence and Roman (1947) found in maize forward growth of pollen tubes which contained heterochromatic B-chromosomes.

Whether euchromatic B-chromosomes can also have an unfavourable effect is not yet known. Still it must be noted that all samples of *T. grandiflorum* that had euchromatic telocentrics were weak and rarely flowered.

6. SUMMARY

1. Plants of *Trillium grandiflorum* and *T. luteum* have been found with one to two small euchromatic fragment chromosomes, the centromere of which is either submedian (standard fragments, *fr*) or terminal (telocentrics).

2. Crosses of the type O × *fr*, *fr* × O and *fr* × *fr* show that distribution of single standard fragments is normal (1 : 1 ratio) if the male plant alone carries the fragment. When it is carried on the female side, however, there is a preponderance of endosperms with two to three fragments (see table 2).

3. It is assumed that there is a directed movement of univalent fragments at meiosis in EMC toward the dyad cell which forms the embryo sac. This would appear to be another mechanism for the maintenance of B-chromosomes in wild populations.

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Plate

Microphotographs of chilled root-tip and endosperm metaphases

FIG. 1.—Metaphase of root-tip cell with standard fragment. ×1000.

FIG. 2.—Metaphase of root-tip cell with telocentric. ×1000.

FIG. 3.—Metaphase of endosperm with one standard fragment. ×900.

FIG. 4.—Metaphase of endosperm with 2 standard fragments. ×900.

FIG. 5.—Metaphase of endosperm with 3 standard fragments. ×900.

FIG. 6.—Hexaploid metaphase of endosperm with 4 telocentrics (2 chromosomes cut). ×500.

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DOUBLE REDUCTION AND NUMERICAL NON-DISJUNCTION IN TETRAPLOID MAIZE

D. G. CATCHESIDE
Department of Genetics, University of Adelaide *

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1. MODES OF SEGREGATION

GENETIC segregation in tetraploids is complicated by modes of gamete formation not found in diploids. For a single locus, there are three of these modes. Besides (1) the contribution to a gamete of one chromatid from each of two chromosomes out of four, there are the additional possibilities of (2) the contribution of two chromatids from one chromosome (Mather, 1936) and (3) the contribution of more or less than two chromatids to a gamete. The second mode is the result of what is usually known as chromatid segregation, in contrast to the normal chromosome segregation, the contribution of two sister chromatids and their genes to one gamete occurring through double reduction. For a particular locus, the frequency with which sister genes are present in the same gamete is measured by the parameter α (Fisher and Mather, 1943). The third mode is a consequence of numerical non-disjunction. This is a factor which cannot be neglected, at least in maize, because a substantial fraction of the progeny of a tetraploid have fewer or more than the tetraploid chromosome number. This has never been properly estimated for a particular chromosome, but is probably of the order of about 5 per cent. for each chromosome in maize.

In all previous genetic studies of tetraploids the effect of non-disjunction has not been separated from that of double reduction which it resembles superficially. This paper is an attempt to estimate the magnitude of these two factors for a particular locus in maize.

2. MATERIAL AND METHODS

The locus studied was sugary-1 (*su*-1) in chromosome 4. In the diploid, by the use of structural changes, this locus has been placed at not less than 9 map units from the centromere and in the shorter arm (Anderson and Randolph, 1945).

All the material was derived from a starchy tetraploid stock donated by Dr L. F. Randolph and a sugary tetraploid donated by Dr Janaki Ammal. Chromosome numbers have been determined in root tips, pretreated with 8-hydroxyquinoline usually in a refrigerator at 5° C. for three to four hours, and fixed in acetic alcohol. Squash preparations were made in acetic orcein after hydrolysis for five minutes in N HCl. The identification of the chromosomes in a somatic metaphase plate can be accomplished fairly reliably by taking into account the ratio of the long to the short arm for each chromosome and the ratio of the total length of the chromosome to the long arm of chromosome 6, which may always be identified by the trabant present on the short arm. The average statistics for identification are shown in fig. 1. It is unfortunate that chromosome IV is not a particularly easy one to identify, but it is believed that the data are reasonably reliable.

* Present address: Department of Microbiology, University of Birmingham.

3. CHROMOSOME NUMBERS OF PROGENY OF TETRAPLOIDS

The chromosome numbers of progeny of parent plants with 40 chromosomes have been determined. They are compared in table 1 with the pooled data collected by Kadam (1944). The two sets of data are consistent with one another.

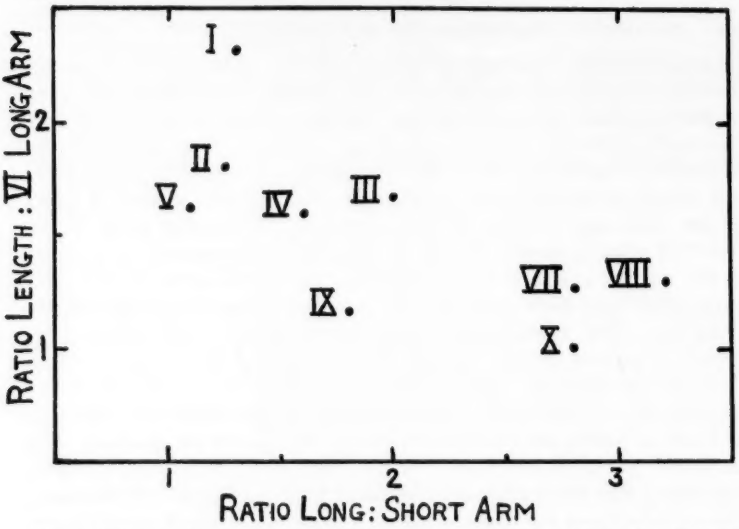

FIG. 1.—Diagram showing the statistics of chromosomes I to V and VII to X, with respect to one another and to chromosome VI.

Taken as a whole the data show 259 chromosomes in excess or deficient in 503 plants. If we assume that only numerically balanced pollen grains function as gametes, there were 259 non-disjunctions amongst 503 eggs, a mean of 0·515 per gamete, or a chance of 0·0515

TABLE 1

Chromosome numbers of progeny of 40 chromosome tetraploid maize

Source	36	37	38	39	40	41	42	43	Totals	Means
(1)	4	1	36	43	**261**	73	8	3	*429*	39·92±0·046
(2)	...	...	3	8	50	13	...	...	*74*	39·99±0·078
Totals	4	1	39	51	**311**	86	8	3	*503*	39·93±0·041

(1) Kadam, 1944. (2) Present paper.

per chromosome per female gamete for non-disjunction. If we assume that numerically unbalanced pollen grains are not handicapped in functioning as gametes, the non-disjunctional chance per chromosome

per female gamete could be as low as 0·02575. Presumably the actual value is somewhere between these limits.

Some indication of the relative likelihood with which non-disjunctional gametes function as eggs and as sperm is given by Punyasingh's (1947) data on the chromosome numbers of tetraploid × diploid and reciprocal crosses. These indicate rates of non-disjunction per chromosome per gamete of 0·0748 amongst eggs and 0·0524 amongst sperm, a ratio of 1·43. Apart from the fact that these values are higher than amongst the progeny of tetraploids, one cannot be sure that the relative performance of gametes in a diploid × tetraploid cross would be exactly similar to that in the tetraploid × tetraploid,

TABLE 2

Representation of the ten different chromosomes (I to X) in progeny of tetraploid maize

(*a*) 74 unselected progeny of 40 chromosome plants

Chromosomes per plant	I	II	III	IV	V	VI	VII	VIII	IX	X	Totals
3	...	1	4	2	...	3	1	2	...	...	*13*
4	73	71	69	70	73	71	73	71	70	73	*714*
5	1	2	1	2	1	...	...	1	4	1	*13*

(*b*) 27 *su*-1 progeny from triplex × nulliplex.

Chromosomes per plant	I	II	III	IV	V	VI	VII	VIII	IX	X	Totals omitting IV
3	...	1	1	9	1	2	2	...	3	1	*11*
4	27	25	26	18	26	25	25	25	23	25	*227*
5	...	1	...	...	...	...	...	2	1	1	*5*

nor that the relative viabilities of unbalanced near triploids would be similar to those of unbalanced near tetraploids. Nevertheless, it would appear reasonable to assume that amongst our material, the ratio of non-disjunction amongst female and male gametes is similar and that the non-disjunctional chance per chromosome per female gamete is about $0{\cdot}0515 \times \frac{1{\cdot}43}{2{\cdot}43} = 0{\cdot}0382$.

During the present observations, attempts were made to determine the numbers of each of the ten different chromosomes. These attempts were by no means exhaustive. Wherever the count departed from 40, the chromosomes were identified to discover which ones departed

from being present in quadruplicate. Where the count was 40, the chromosomes were identified to see that there were no departures from there being four of chromosome VI, 20 chromosomes longer than VI (numbers I to V) and 16 chromosomes shorter than VI (numbers VII to X). In two cases, the number of 40 was found to be unbalanced, being internally compensated through five of one chromosome making up the deficit due to three of another chromosome. No doubt other internal compensations escaped detection.

The results are given in table 2, separately for unselected plants and plants which, arising from the triplex backcross, showed the recessive sugary-1. The unselected plants show a mean of 0·0352 non-disjunctions per chromosome per plant; the selected ones, omitting chromosome 4, show a mean of 0·0684. The values are not significantly different, and the weighted mean is 0·0432. It will be noticed that a third of the selected plants have only three of chromosome IV (plate, fig. 2) and it is reasonable to conclude, as discussed in the next section, that each of these owed its recessive phenotype to non-disjunction in the formation of the female gamete, rather than to double reduction.

4. SEGREGATION RATIOS IN TETRAPLOIDS

In a tetraploid organism, there are three possible sorts of heterozygote for a given digenic locus, respectively with one, two or three doses of a recessive gene. These heterozygotes are usually known as triplex, duplex and simplex heterozygotes, from the presence of three, two and one dominant genes. The regular gametes formed contain two of each sort of chromosome and with regular chromosome segregation the simple gametic ratios given in the first section of table 3 are expected. In the case of a locus not situated at the centromere of the chromosome, a proportion of double reduction may be expected as a result of crossing-over between the locus and the centromere, followed by non-disjunction of the sister genes at both the first and second division of meiosis. If the coefficient of such double reduction is α, the gametic ratios shown in the second part of table 3 are expected.

Besides regular segregation in a tetraploid, numerical non-disjunction of the chromosomes may occur, resulting in gametes receiving either three or one of a particular kind of chromosome instead of the usual two. If numerical non-disjunction occurs in a proportion x of meiotic cells, so that a similar proportion of gametes shows the effects of such non-disjunction, the gametic ratios shown in the third part of table 3 may be expected if the complication of numerical non-disjunction is simply added to chromosome segregation. If chromatid segregation and numerical non-disjunction are both assumed to occur in a tetrasomic organism the gametic ratios shown in the fourth part of table 3 may be expected, if the two events do not occur together in a cell.

TABLE 3

The effects of double reduction and numerical non-disjunction upon the proportion of gametes of different constitutions formed by tetrasomic heterozygotes

Type :		Triplex	Duplex	Simplex
Constitution :		*AAAa*	*AAaa*	*Aaaa*
Gametes formed by Chromosome Segregation	*AA*	$\frac{1}{2}$	$\frac{1}{6}$	...
	Aa	$\frac{1}{2}$	$\frac{2}{3}$	$\frac{1}{2}$
	aa	...	$\frac{1}{6}$	$\frac{1}{2}$
Gametes formed with Chromatid Segregation (*Double Reduction*)	*AA*	$\frac{2+\alpha}{4}$	$\frac{1+2\alpha}{6}$	$\frac{\alpha}{4}$
	Aa	$\frac{1-\alpha}{2}$	$\frac{2-2\alpha}{3}$	$\frac{1-\alpha}{2}$
	aa	$\frac{\alpha}{4}$	$\frac{1+2\alpha}{6}$	$\frac{2+\alpha}{4}$
Gametes formed with Numerical Non-disjunction	*AAA*	$\frac{x}{8}$	...	...
	AAa	$\frac{3x}{8}$	$\frac{x}{4}$	...
	Aaa	...	$\frac{x}{4}$	$\frac{3x}{8}$
	aaa	...	...	$\frac{x}{8}$
	AA	$\frac{1-x}{2}$	$\frac{1-x}{6}$	...
	Aa	$\frac{1-x}{2}$	$\frac{4(1-x)}{6}$	$\frac{1-x}{2}$
	aa	...	$\frac{1-x}{6}$	$\frac{1-x}{2}$
	A	$\frac{3x}{8}$	$\frac{x}{4}$	$\frac{x}{8}$
	a	$\frac{x}{8}$	$\frac{x}{4}$	$\frac{3x}{8}$
Gametes formed with Double Reduction and Numerical Non-disjunction	*AAA*	$\frac{x}{8}$	...	...
	AAa	$\frac{3x}{8}$	$\frac{x}{4}$	...
	Aaa	...	$\frac{x}{4}$	$\frac{3x}{8}$
	aaa	...	...	$\frac{x}{8}$
	AA	$\frac{(2+\alpha)(1-x)}{4}$	$\frac{(1+2\alpha)(1-x)}{6}$	$\frac{\alpha(1-x)}{4}$
	Aa	$\frac{(1-\alpha)(1-x)}{2}$	$\frac{(2-2\alpha)(1-x)}{3}$	$\frac{(1-\alpha)(1-x)}{2}$
	aa	$\frac{\alpha(1-x)}{4}$	$\frac{(1+2\alpha)(1-x)}{6}$	$\frac{(2+\alpha)(1-x)}{4}$
	A	$\frac{3x}{8}$	$\frac{x}{4}$	$\frac{x}{8}$
	a	$\frac{x}{8}$	$\frac{x}{4}$	$\frac{3x}{8}$

Strictly speaking, the expectations in the fourth part of table 3 need correcting in respect of non-disjunctional gametes in which double reduction has occurred. This will affect only those gametes which receive three allelic genes. The consequential corrections are as follows :

Gamete	Triplex	Duplex	Simplex
AAA	$\frac{x(1+\alpha)}{8}$	$\frac{\alpha x}{12}$	
AAa	$\frac{3x-2\alpha x}{8}$	$\frac{x(3-\alpha)}{12}$	$\frac{\alpha x}{8}$
Aaa	$\frac{\alpha x}{8}$	$\frac{x(3-\alpha)}{12}$	$\frac{x(3-2\alpha)}{8}$
aaa		$\frac{\alpha x}{12}$	$\frac{x(1+\alpha)}{8}$

It will be noticed that chromatid segregation and numerical non-disjunction each contribute to the presence of gametes bearing only recessive genes, for the locus in question, when none are expected (the triplex heterozygotes) and also to a greater frequency of such gametes than are expected on purely chromosome segregation (the duplex and simplex heterozygotes). The expectations of recessives are $\frac{x}{8}+\frac{\alpha(1-x)}{4}$ from a triplex heterozygote, $\frac{2+x}{12}+\frac{\alpha(1-x)}{3}$ from a duplex and $\frac{1}{2}+\frac{\alpha(1-x)}{4}$ from a simplex. In the case of the triplex the expectation is approximately $\frac{x+2\alpha}{8}$, for $\frac{\alpha x}{4}$ is relatively negligible. The value of x in this case is not simply the proportion of non-disjunction, but includes a factor, not separately estimated, dependent upon the fact that an unbalanced gamete (and zygote), arising from non-disjunction, is less viable than a balanced one. A difference between the viability of gametes (and zygotes) with one chromosome short or in excess could be expected, but has been assumed absent in the present discussion.

Very small corrections to the duplex and simplex expectations should be applied if the formation of three gene double reductional gametes is taken into account. The exact expectations are $\frac{2+x}{12}+\frac{\alpha(4-x)}{12}$ for duplex and $\frac{1}{2}+\frac{\alpha(2-x)}{8}$ for simplex. The less exact expectations have been used in the following calculations, mainly because they are simpler to apply and display (table 5). However, the results of calculations using the more exact formulæ will be given.

The available information may be used in two ways to yield the parameters x and α. In the first place, we may consider jointly the

progeny of the three sorts of heterozygote, for which the expectations and observations, pooled from a large number of families, are given in table 4. The maximum values of the parameters, each on the assumption that the other is zero, may be estimated using Fisher's (1946) method of scores. This yields $\alpha = 0{\cdot}0377 \pm 0{\cdot}0059$ and $x = 0{\cdot}0825 \pm 0{\cdot}0136$. The value of x is well above what is plausible in view of the cytological data on rates of chromosome non-disjunction previously considered. We may therefore conclude that α does not take a zero value. On the other hand, there is no *a priori* expectation as to the maximum value of α for any particular locus, though it is

TABLE 4

Observed segregations in backcross progeny of tetrasomics, heterozygous for sugary-1, used as seed plants

Heterozygote	Progeny	Observed	Expected proportions
TRIPLEX . .	*su*-1$^+$	3326	$1 - \frac{x}{8} - \frac{\alpha(1-x)}{4}$
	su-1	33	$\frac{x}{8} + \frac{\alpha(1-x)}{4}$
DUPLEX . .	*su*-1$^+$	5110	$\frac{5}{6} - \frac{x}{12} - \frac{\alpha(1-x)}{3}$
	su-1	1115	$\frac{1}{6} + \frac{x}{12} + \frac{\alpha(1-x)}{3}$
SIMPLEX . .	*su*-1$^+$	3078	$\frac{1}{2} - \frac{\alpha(1-x)}{4}$
	su-1	3126	$\frac{1}{2} + \frac{\alpha(1-x)}{4}$

possible to make plausible guesses by the means discussed later, provided various other cytological and genetical parameters are known.

Maximum likelihood estimates of the parameters may be obtained jointly from the observed numbers. This is done by partial differentiation, for the two parameters in question, followed by the construction of scores and information per plant scored. The various items in the estimation and the values of the scores and information in a late round of iteration are given in table 5. It is clear that the estimates are not very precise, though the limits would not be symmetrically arranged about the estimates, because the probabilities of the events x and α are small.

After the parameters had been computed in this way, they were given a trial in the more exact formulæ, which take account of three gene, double reductional gametes. After several iterations this gave $x = 0{\cdot}03380$, $V_x = 0{\cdot}0019015$, $\alpha = 0{\cdot}02382$, $V_\alpha = 0{\cdot}00037295$.

These estimates of x and α may now be used to calculate, for each of the kinds of progeny, numerical expectations which may be compared with the observations. The comparison yields a χ^2, for one

degree of freedom, of 0·235778, for which P is between 0·5 and 0·7. Thus the data are fitted very well by these estimates of the parameters.

The second method of estimation depends upon a joint genetical and cytological examination of the progeny of the triplex backcrosses. Amongst these there were 33 sugary seeds in the grand total of 3359.

TABLE 5

(a) *Scores and information for joint estimation of* x *and* α

Class	Progeny	x scores	α scores	i_{xx}	$i_{x\alpha}$	$i_{\alpha\alpha}$
Triplex	*su*-1$^+$	$-\frac{1-2\alpha}{8-t}$	$-\frac{2(1-x)}{8-t}$	$\frac{(1-2\alpha)^2}{t(8-t)}$	$\frac{2(1-2\alpha)(1-x)}{t(8-t)}$	$\frac{4(1-x)^2}{t(8-t)}$
	su-1	$\frac{1-2\alpha}{t}$	$\frac{2(1-x)}{t}$			
Duplex	*su*-1$^+$	$-\frac{1-4\alpha}{10-u}$	$-\frac{4(1-x)}{10-u}$	$\frac{(1-4\alpha)^2}{(2+u)(10-u)}$	$\frac{4(1-4\alpha)(1-x)}{(2+u)(10-u)}$	$\frac{16(1-x)^2}{(2+u)(10-u)}$
	su-1	$\frac{1-4\alpha}{2+u}$	$\frac{4(1-x)}{2+u}$			
Simplex	*su*-1$^+$	$\frac{\alpha}{2-v}$	$-\frac{1-x}{2-v}$	$\frac{\alpha^2}{(2-v)(2+v)}$	$-\frac{\alpha(1-x)}{(2-v)(2+v)}$	$\frac{(1-x)^2}{(2-v)(2+v)}$
	su-1	$-\frac{\alpha}{2+v}$	$\frac{1-x}{2+v}$			

$t = x+2\alpha-2\alpha x$; $u = x+4\alpha-4\alpha x$; $v = \alpha-\alpha x$.

(b) *Computations for* x = *0·03213*, α = *0·02463*

Class	Progeny	x scores	α scores	I_{xx}	$I_{x\alpha}$	$I_{\alpha\alpha}$
Triplex	*su*-1$^+$	−399·2532	−812·8935	4803·3095	9779·7050	19911·8255
	su-1	393·1138	800·3936			
Duplex	*su*-1$^+$	−466·6050	−2003·8737	240·8554	1034·3734	4422·2002
	su-1	472·4582	2029·0108			
Simplex	*su*-1$^+$	38·3629	−1507·5215	0·9410	−36·9795	1453·1404
	su-1	−38·0432	1494·9608			
Totals		0·0335	0·0765	5045·1059	10777·0989	25807·1661

$x+\delta x = 0{\cdot}03213(2837)$ $\quad V_x = 0{\cdot}00183628$
$\alpha+\delta\alpha = 0{\cdot}02463(1759)$ $\quad V_\alpha = 0{\cdot}00035897$

Efforts were made to determine the numbers of chromosome IV present in each of the sugary progeny. The results (table 2*b*) for 27 which were successfully analysed showed 9 which each had only three of chromosome IV in their somatic complements. This is altogether a very much higher frequency of non-disjunction than has been encountered for any other chromosome and it is very probable

that the egg from which each of these trisomic tetraploids was formed had only one chromosome IV, and that this single chromosome carried the *su*-1 gene. The alternative explanation would be that the pollen parent introduced a single chromosome IV into the zygote. In this case the zygote must owe its sugary phenotype to double reduction in the formation of the egg. This would imply an unreasonably high frequency of functioning of male gametes deficient for one chromosome IV unless there were preferential fertilisation by them of eggs showing double reduction. In what follows it will be assumed that the 9, each with only three of chromosome IV, owe their origin to non-disjunction in egg formation and that the 18, each with four of chromosome IV, owe their origin to double reduction in egg formation.

We have then that the proportion of the triplex recessive gametes that arises through non-disjunction is one-third. The limit values,

TABLE 6

Rates of non-disjunction (x), *double reduction* (α) *and* $y = \alpha(1-x)$ *for the sugary locus in tetraploid maize, using combined genetical and cytological analysis of triplex heterozygotes*

Parameter	Experimental value	Upper limit $p = 0{\cdot}05$	Lower limit $p = 0{\cdot}05$
x	0·0262	0·0424	0·0130
y	0·0262	0·0328	0·0181
α	0·0269	0·0332	0·0189

probable at the 0·05 level, may be calculated with the aid of Steven's tables (1942). Using these numbers multiplied by a factor $\frac{33}{27}$, to take account of the fact that not all the sugary progeny were analysed cytologically, and by factors of 8 and 4 respectively, the upper and lower limits of x and y, $= \alpha(1-x)$, and thence α may be calculated. The results are given in table 6. The results of this method agree very well with those given by the first, purely genetic, method.

5. ESTIMATION OF THE PROPORTION (*r*) OF RECESSIVE GAMETES PRODUCED BY TRIPLEX HETEROZYGOTES

A triplex heterozygote may often be distinguished from a quadruplex (dominant homozygote) by its production of one or more recessive (nulliplex) offspring when pollinated by a nulliplex and always by the presence of simplex offspring in this same progeny. If no recessive offspring appear, leaving it uncertain whether the parent was quadruplex or triplex, the first backcross progeny must be tested by a second backcross to detect the presence of simplex plants or establish their absence with a given reasonable level of

probability to conclude that the original test plant was quadruplex rather than triplex. This second backcross test is laborious and time-consuming and has not been performed upon all the plants from which the data included here have been collected.

Some of the triplex plants used appeared in the progeny of selfed duplex plants. In this case, those plants which might have been quadruplex or triplex were tested by a second backcross. However, most of the triplex plants appeared in crosses between quadruplex and simplex plants, in which the majority of the offspring would be triplex or duplex, in equal proportion, and only a minority quadruplex through the occurrence of double reduction in the simplex parent. It has been assumed in this case that all families showing no recessives were in fact from triplex plants.

There were 33 *su* seeds among a total of 3359, or just under 1 per cent. The distribution of these recessives amongst the 24 families of triplex progenies is given in table 7.

TABLE 7

Progenies from tetraploid maize, triplex for su-1$^+$, *pollinated by nulliplex*

Number *su*	Sizes of progenies
0	184, 39, 105, 165, 170, 46, 65
1	211, 206, 76, 74, 18
2	259, 84, 65, 219, 86, 188, 131, 173, 181
3	251, 140
4	223

Mr G. N. Wilkinson (C.S.I.R.O. Division of Mathematical Statistics) has shown me that it is possible to obtain satisfactory estimates of the frequency of the recessives, omitting those families which lack any recessive. When dealing with truncated binomial probabilities of this description the expectation of a given family, among those considered (which must have at least one recessive) is

$$\frac{{}_nC_r p^r (1-p)^{n-r}}{1-(1-p)^n}.$$

Maximum likelihood estimates of the proportion of recessives, and of their variance may be derived. Their computation involves an elegant mode of iteration, by means of reciprocals, that Mr Wilkinson will be describing elsewhere.

From the present point of view, it is interesting to note that the estimate of the size of the population sample containing the 33 *su* seeds is 3401·7, and leads to a parameter not significantly different from the value (33/3359) obtained on the assumption that all the zero class families in table 7 should be included in the estimate.

6. SEGREGATION RATIOS IN PLANTS TRISOMIC OR PENTASOMIC FOR A CHROMOSOME

Progeny which are trisomic or pentasomic may be expected to occur as a result of numerical non-disjunction in a tetrasomic plant. They may be detected, in some instances, by the special segregations shown when heterozygous for suitable markers. Two kinds of heterozygote, *AAa* and *Aaa*, are possible in the trisomic, and four kinds, *AAAAa*, *AAAaa*, *AAaaa* and *Aaaaa*, in the pentasomic.

The trisomics will usually form gametes with one or two of these particular chromosomes and it is probable that those gametes with but one of these chromosomes and resulting zygotes with three (instead of four) would be less viable than those with balanced numbers.

TABLE 8

Segregation ratios in trisomic and pentasomic plants, with the viabilities of trisomic and pentasomic progeny respectively u *and* v

Trisomics	Gametes					Divisor	Ratios in backcross progeny
	AA	*Aa*	*aa*	*A*	*a*		*A* : *a*
AAa	1	2	...	2*u*	*u*	6	3+2*u* : *u*
Aaa	...	2	1	*u*	2*u*	6	2+*u* : 1+2*u*

Pentasomics	Gametes							Divisor	Ratios in backcross progeny
	AAA	*AAa*	*Aaa*	*aaa*	*AA*	*Aa*	*aa*		*A* : *a*
AAAAa	2*v*	3*v*	...	...	3	2	...	10	1 : 0
AAAaa	*v*	6*v*	3*v*	...	3	6	1	20	9+10*v* : 1
AAaaa	...	3*v*	6*v*	*v*	1	6	3	20	7+9*v* : 3+*v*
Aaaaa	...	...	3*v*	2*v*	...	2	3	10	2+3*v* : 3+2*v*

Similarly, it is probable that the unbalanced gametes and zygotes with excess numbers, as in the pentasomic and its pentasomic progeny, would also be less viable than the balanced types. If the viability of the trisomic is *u* and of the pentasomic is *v*, the expectations with chromosome segregation are those shown in the two parts of table 8. With the great uncertainty introduced by the viability factor, it is hardly worth while considering any correction due to double reduction. The ratio of *A* to *a* in backcross progeny would vary with the value of *u* and *v*, according to the ways indicated in fig. 3. It will be noticed that *Aaa* and *Aaaaa* may be confused with *Aaaa* and *AAa* with *AAaa* if *u* has a high value ; *AAAAa* will be confused with *AAAa* under all circumstances, while *AAAaa* and usually *AAaaa* seem recognisable whatever value *v* might take.

One backcross family consisted of 160 *su*+ and 57 *su*, a very bad agreement with the 5 : 1 ratio expected in a duplex backcross ($\chi^2 = 14{\cdot}401$). The plant which gave this family was assumed to be a duplex pentasomic, namely $+ + su\ su\ su$. The viability, v, of the pentasomic, may be estimated as 0·229, and we should expect a fraction $\frac{9v}{7+9v}$, or 0·22746, of the dominant heterozygotes to be pentasomic, one-third of them duplex and two-thirds simplex. A

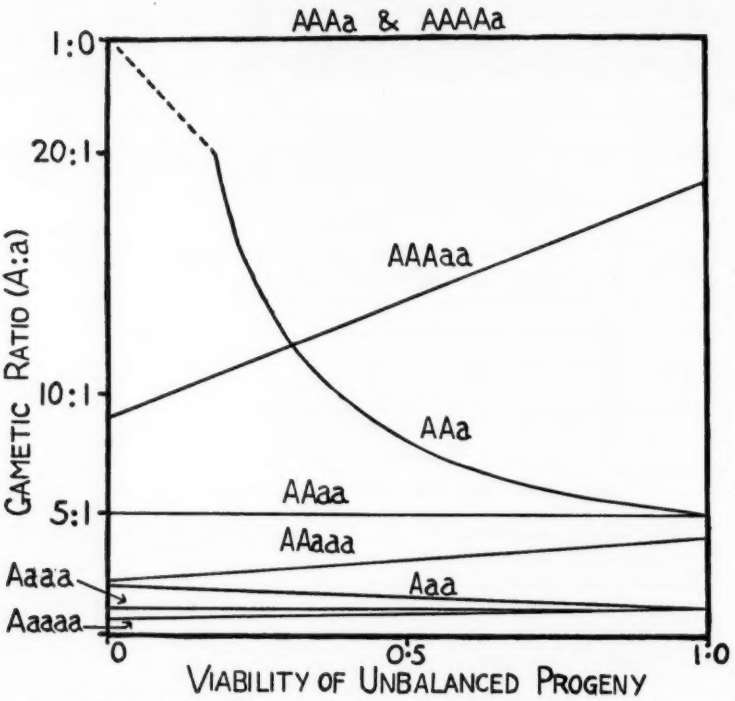

FIG. 3.—Relation between the expected gametic ratios of trisomic and pentasomic tetraploids and the viabilities of their trisomic and pentasomic progeny.

number of the dominant heterozygotes were planted. The chromosome number was counted in 39 plants and backcross progenies were obtained from every one of these. Two plants each had five chromosomes IV and their backcross progenies showed 45+ : 50 *su* and 94+ : 106 *su*, respectively. Evidently these two plants were $+\ su\ su\ su\ su$. If indeed the original parent were pentasomic we should expect the 39 plants tested to consist of 4·3 $+ + su\ su$, 25·82 $+\ su\ su\ su$, 2·96 $+ + su\ su\ su$ and 5·92 $+\ su\ su\ su\ su$. The observed numbers were 7, 30, 0 and 2. The χ^2 for three degrees of freedom is 7·9255

and P is 0·02-0·05. If the original plant had been tetrasomic, the duplex and simplex progeny would have been expected in the ratio of 1 : 4. While the results do not permit a perfectly definite conclusion, they are distinctly unfavourable to the assumption that the original plant was pentasomic. The two progeny that were pentasomic for chromosome IV could conceivably be fresh cases of numerical non-disjunction.

7. DISCUSSION

There can be no doubt that non-disjunction makes an appreciable contribution to gamete formation and that the effects of it have previously been confused with the effects of double reduction. This means that the rates of double reduction have been overestimated, particularly for loci close to the centromere. No other data suitable for analysis in the ways investigated above appear to be present in the literature. Frequently, one of the types of mating is missing from the data. In other cases, the numbers of progeny that have been enumerated are too small. Finally, some sets of tetrasomic segregation data show evidence of differential viability, which tend to swamp the effects due to non-disjunction and double reduction. In no other case has it been possible to differentiate, amongst the progeny of triplex heterozygotes, those with three or four of the chromosome concerned.

It is interesting to consider how far the estimate of double reduction determined above agrees with the value expected from the interaction of the various contributory factors. The statistics for the cytological factors are largely based on data in a Cambridge Ph.D. thesis written by Dr Jillela Venkateswarlu, to whom I am indebted for permission to use the relevant material.

The coefficient of double reduction, α, is dependent upon the following coefficients : (1) the amount of crossing-over (c) between the centromere and the locus ; (2) the frequency (q) of quadrivalent formation ; (3) the frequency (p) of adjacent (or parallel) disjunction of quadrivalents ; and (4) the frequency (d) with which adjacent disjunction of the quadrivalent results in non-disjunction of the genes in paired chromosome arms. These parameters are related by the formula $\alpha = cqpd$, since the half chance of having the necessary disjunctional arrangement at division II of meiosis is offset by the double chance of the necessary cross-over in each cell. Dr Venkateswarlu's data indicate that the value of q, averaged for all chromosomes (I to X), is 0·769 and the value of p is 0·373. The value of d may be taken as 0·5, in the absence of any evidence of how the difference in lengths of chromosome arms affects the chances of the two parallel arrangements. Thus in maize the average relation would be $\alpha = 0{\cdot}769 \times 0{\cdot}373 \times 0{\cdot}5 \times c = 0{\cdot}1434c$. For the sugary-1 locus, c is not less than 0·09 (Anderson and Randolph, 1945) and this would indicate 0·0129 as a minimum value for α. Alternatively, from our measurement of α, we could argue that the value of c is closer to 0·19.

It would be useful to obtain information of this nature for a series of loci at different positions with respect to the centromere. We should expect that the contribution due to non-disjunction would be constant for a given chromosome, and that α would rise from a zero value at the centromere to a maximum of $\frac{1}{7}$, except that this might be exceeded if certain conditions of interference were fulfilled. The maximum value of one-seventh could be achieved only if q were unity and p were one-half.

8. SUMMARY

1. There are three components of variation in tetraploid segregation, *viz.* chromosome and chromatid segregation, and numerical non-disjunction. The two latter components have not been discriminated from one another in previous studies, but it has been possible to separate all three in an experiment with the sugary-1 locus in chromosome 4 of tetraploid maize. The ten different chromosomes can be distinguished at mitosis, and unbalanced complements have been demonstrated in many plants.

2. The coefficient of double reduction (α) at the sugary-1 locus is approximately 2·7 per cent. The coefficient of non-disjunction (x) is about 2·6 per cent., when estimated from triplex backcross progeny, amongst which the recessives are analysed cytologically. Estimates made from triplex, duplex and simplex data treated together give maximum likelihood estimates of $\alpha = 2{\cdot}4$ per cent. and $x = 3{\cdot}4$ per cent.

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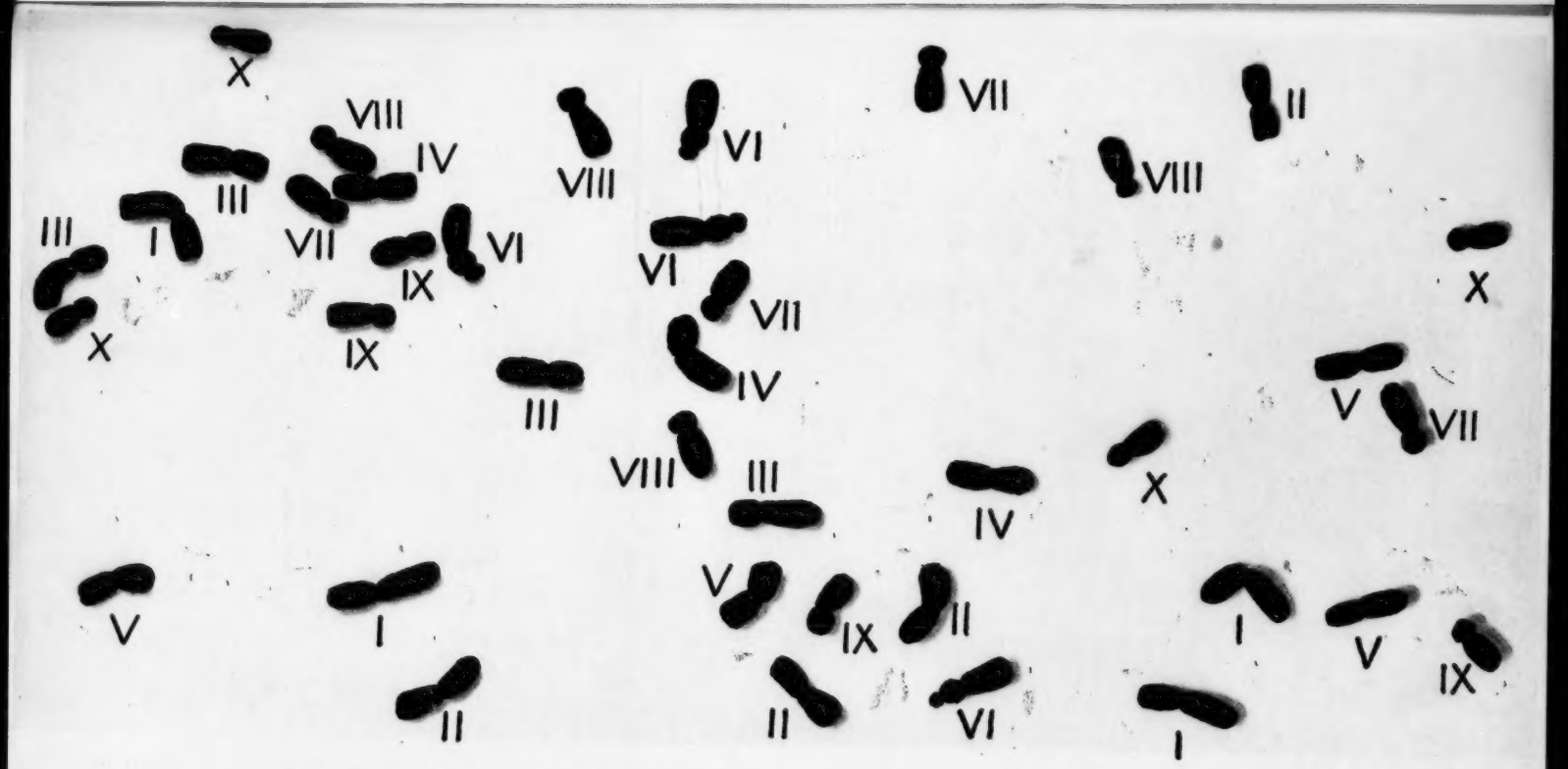

FIG. 2.—The somatic chromosome complement of a 39 chromosome plant having only three of chromosome IV.

HETEROSTYLY AND HOMOSTYLY IN *PRIMULA OBCONICA*

V. PAMELA J. DOWRICK

John Innes Horticultural Institution, Bayfordbury, Hertford, Herts

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I. INTRODUCTION

THE complex of characters, both morphological and physiological, which distinguishes the two mating types of most distylic species of plants, is well known. The thrum plant has flowers with short styles, high anthers, large pollen and short stigmatic papillæ; while the pin has long styles, low anthers, small pollen and long papillæ. The two forms of pollen and style differ physiologically, so that pollinations within mating types are incompatible, but between two types are compatible.

The genetic control of distyly is also remarkably similar in most species. One gene complex controls the difference, thrum being a heterozygote, **Ss** and pin the recessive homozygote, **ss**. In autotetraploids of *Primula sinensis* the expression and segregation of the **S** complex is known to be quite regular. The simplex form, **Ssss**, is a thrum, thus showing the complete dominance of **S**.

Abnormal plants which show combinations of some thrum and some pin characters have been found in many species. Certain of these abnormalities have the style and anthers at the same level, and are described as *homostyles*. In *P. sinensis* the homostyles are the result of mutant genes at loci other than that of the **S** complex (Mather, 1950). However, in *Primula viscosa* and *Primula hortensis* (Ernst, 1936*b*), and in *Primula vulgaris* (Crosby, 1949*a*), the homostyles are the result of a change in the **S** complex itself. It has not been proved whether this change is the result of a mutation of one or more of the **S** subgenes or of crossing-over within the **S** complex.

Primula obconica, the subject of this study, is a typical distylic species which exists in both diploid and autotetraploid forms. Homostyles have not been found in the diploids but are present in the tetraploids.

The main objects of this investigation were to study:

1. The morphological and physiological characters controlled by the **S** complex in diploid and tetraploid *P. obconica*.

2. The inheritance of the **S** complex in both forms and the genetics of the homostyle tetraploid.

3. The structure and behaviour of the **S** complex; the mode of its breakdown, and the relationship if any between breakdown of the **S** complex and polyploidy.

2. MATERIAL AND METHODS

Diploid and autotetraploid horticultural varieties of *P. obconica* were used.

Pollen grains, stigmatic papillæ and conducting tissue were stained in Cotton Blue, hand sections being used for the last named tissue.

Compatibility was tested initially by pollen-tube growth in the style. Flowers were removed from the plants, pollinated, and kept in an incubator at 25° C. for twenty-four hours. Styles were then removed from the flowers and fixed in 1 : 3 acetic alcohol for twenty-four hours and stained in acid fuchsin and light green (Darlington and La Cour, 1947). They were softened in water, halved longitudinally, and squashed before warming in stain for ten minutes.

Although normally grown under glass, large progenies of the tetraploid were raised by sowing under glass in January and planting outside at the end of May. A sufficient proportion flowered under outdoor conditions.

I am grateful to Messrs J. T. Jansen of Hertford and H. Owens of Bath for gifts of plants of *P. obconica*.

3. CHARACTERS CONTROLLED BY THE S COMPLEX

(1) *Morphological*

As in other *Primula* species, the diploid pins have long styles, low anthers, long stigmatic papillæ and small pollen grains, when compared with thrums. The tetraploid pin and thrum have similar

TABLE 1

The morphology of distyly in diploid and tetraploid P. obconica

Ploidy	Thrum			
	Style length	Anther height	Stigmatic papilla length	Pollen grain volume
2	5·0	8·7	8·26	3750
4	4·9	9·5	12·78	7300
	Pin			
2	9·5	4·2	28·70	1650
4	11·2	4·5	45·96	3100
	long-styled homostyle			
2	...	...	...	...
4	10·8	9·5	42·56	6400

Style length and anther height are measured in mm. from the level of corolla abscission, stigmatic papilla length in μ ; and pollen grain volume in μ^3.

differences as shown by the measurements given in table 1. Measurements of the tetraploid homostyle showed that the style is of pin length and stamens are at the thrum level ; pollen is large, *i.e.* of the thrum type and stigmatic papillæ are long like those of a pin stigma.

A further difference between pin and thrum has been noted in both diploid and tetraploid *P. obconica*. The cross thrum × pin is more fertile than the reciprocal cross (table 2).

TABLE 2

The comparative fertility of reciprocal crosses in P. obconica

Ploidy	Pin × thrum	Thrum × pin
2	64·9	106·1
4	69·4	89·2

Fertility is measured by the mean seed-set per capsule.

It is interesting that Darwin (1862 and 1877) discovered the same relationship in other *Primula* species, and that *P. sinensis* data from other sources are in agreement (see table 3). In his "Forms of Flowers", Darwin states that, "the short-styled plants produce more seed than the long-styled, in *P. veris* and other species of *Primula*".

TABLE 3

Comparison of the fertility of reciprocal compatible crosses in Primula

	Hildebrand (1864)		Darwin (1862 and 1877)		Mather and de Winton (1941)	
♀ Parent / Species	ss	Ss	ss	Ss	ss	Ss
P. auricula (after Kerner)			73	98		
P. elatior			46·5	47·7		
P. sinensis	41	44	50	64	17·8	30·0
P. veris			(1) *34* (2) *62·8*	*41* *44·5*		
P. vulgaris			(1) *20* (2) 66·9	*40* 65·0		

Fertility is measured by mean number of seeds per capsule and, for figures in italics, by the mean weight in grains per 100 capsules. These weights probably underestimate the difference in fertility since Darwin recorded that pin ovules of *P. veris* were larger than thrum. The second values for *P. veris* and *P. vulgaris* are the result of artificial pollinations. It must be assumed that these are not truly representative, since Darwin noted that seed production was greater in the thrum for these species.

Ernst, too, has noted such a difference. Thus it appears that differences in fertility (seed production), must be included as a feature of dimorphism in *Primula* species.

This difference in fertility was investigated further in *P. obconica*. Pin and thrum ovaries show no difference in the number of ovules

they contain, which is about double the number of seeds set. Therefore, this cannot be a limiting factor in seed production. There must, then, be some other factor to account for a reduced seed set in pin forms. Measurements show that there is a much smaller area of conducting tissue available for pollen-tube growth in pin than in thrum styles (see table 4).

The larger size of thrum pollen does not result in significantly wider pollen tubes, but is presumably correlated with the extra growth in tube length. (If they are wider, then this factor would further reduce the amount of pollen able to grow down the pin styles.) Thus, once it is shown that pollen tube growth is restricted, then the diameter of the conducting strand must be the factor governing the difference in seed production.

There is, in fact, a physical limit to the number of tubes which can grow down a style, those which are " crowded out " giving rise to a secondary zone of short tubes, unable to continue growth. Modlibowska (1942), showed that in *P. obconica*, " *particularly in the cross pin* × *thrum* " there was bimodality of pollen-tube growth after

TABLE 4

Area of cross section of conducting tissue in the style measured in μ^2

Ploidy	Thrum	Pin
2	284·8	127·5
4	305·5	163·8

heavy pollination. This was not due to the two genotypically different forms of thrum pollen, **S** and **s**, since it did not appear after restricted pollination. That bimodality was most striking in pin styles is to be expected as the available area is smaller.

Thus, another morphological difference exists between the two forms in *P. obconica*, and is, most probably, present in other *Primula* species. Whether this is to be regarded as distinct from the style-length character, or as another effect of the same basic difference, is, at present, unknown.

(ii) *Physiological*

Incompatibility is the result of physiological differences in the styles and pollen of the two forms of plant. These are controlled by the **S** complex. In the diploid *P. obconica*, as with other heterostyled species, thrum × pin and pin × thrum are the compatible unions, there is a full seed set and the pollen tubes grow 3 mm. in 24 hours. After self-pollination no seeds are produced and pollen tubes rarely penetrate the stigma in 24 hours.

Incompatibility behaviour of the tetraploid thrum and pin was practically identical with the diploid. Self-pollination of pin plants did, however, sometimes give a number of long pollen tubes after 24 hours and set small quantities of seed. Thus in tetraploids the incompatibility reaction is not as rigorous as in diploids. Pollen-tube growth studies of the tetraploid long homostyle showed that the incompatibility reaction of the style was "pin" and the pollen behaved as "thrum" (see fig. 1). Thus, homostyle × thrum and pin × homostyle were compatible crosses; the reciprocal crosses were incompatible, and the homostyle was self-fertile.

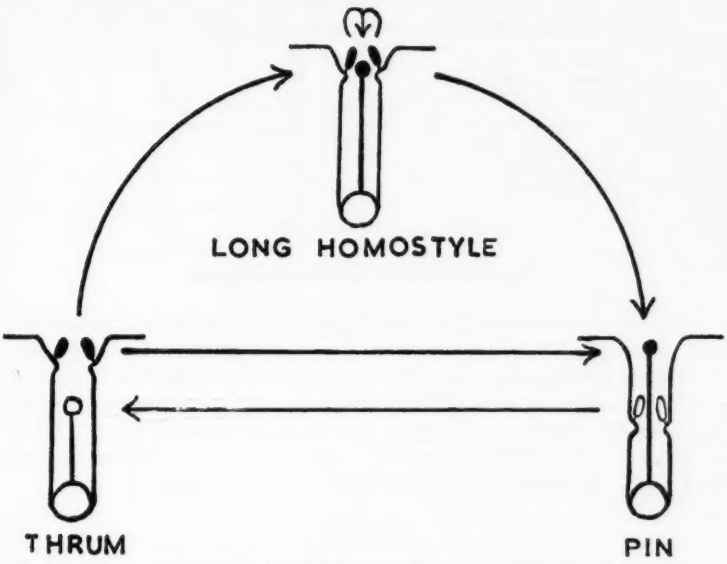

FIG. 1.—Compatibility relationships of the three forms of tetraploid *P. obconica*. Similar shading and arrows indicate the compatible unions.

4. THE INHERITANCE OF THE S COMPLEX

In diploid *P. obconica*, as in other heterostyled species, the S gene complex is inherited as a single gene pair, **S-s**. Thrum is the dominant form and is heterozygous, **Ss**; pin is the homozygous recessive, **ss**. There is sporophytic control of the pollen so that both genotypes of thrum pollen have the same compatibility behaviour.

In the tetraploid form reciprocal crosses were made between thrum and pin plants. The progeny approximated to 1 : 1 ratios of the two forms (see table 5). Therefore, the thrum plants must have been simplex for **S**, and furthermore the **S** allele shows complete dominance, as in *P. sinensis*.

It should be pointed out that, in a normal equilibrium population of tetraploid pins and thrums, all the thrums will be simplex. In whatever way the tetraploid thrum arose from the diploid, since it is strictly self-incompatible, it can only reproduce by crossing with the recessive pin. In this way forms containing more than one **S** allele would disappear and the simplex thrums remaining would maintain a population of equal numbers of simplex thrums and pins.

TABLE 5

Inheritance of the **S** *complex in the tetraploid form*

	Thrums	Pins
Thrum (1) × pin	36	46
Thrum (2) × pin	66	71
Thrum (3) × pin	90	85
Pin × thrum (1)	20	23
Pin × thrum (2)	155	157
Pin × thrum (3)	61	77
Total	428	458

The above results all agree with 1 : 1 ratios.

Crosses were also made to investigate the inheritance of homostyly (see table 6). The first cross shows that the homostyle does not carry the thrum allelomorph—**S**. The second, indicates that it is duplex, either for a distinct gene (**HHhhssss**, where **H** is epistatic over **s** but not over **S**), or for an allele of the **S** complex ($\mathbf{S^1S^1ss}$, $\mathbf{S^1}$ being recessive to **S** but dominant over **s**). The 5 : 1 ratio in the first agrees with either hypothesis, as does the 35 : 1 ratio from selfing.

TABLE 6

The inheritance of the tetraploid long homostyle form

Parents \ Progeny classes	Thrum	Homostyle	Pin	Ratio
(1) Pin × homostyle	0	57	12	0 : 5 : 1
(2) Homostyle × thrum	44	36	11	6 : 5 : 1
(3) Homostyle selfed	0	99	5	0 : 35 : 1

Thus homostyly in this species could be either of the kind found in *P. sinensis*, or of that found in *P. hortensis*, *P. viscosa* and *P. vulgaris*. To distinguish between these two possibilities it was necessary to analyse thrum plants obtained from the cross, long homostyle × thrum

(see table 7). Nineteen of these, taken at random, were back crossed and the progeny scored (see table 8).

The results, when taken together, fit the ratios expected on the **S** allele theory. Individual families, too, fit these ratios, with the exception of 246[1] progeny, which fits the 2 : 1 : 1 ratio slightly better, and that of 246[2] which fits neither. Therefore, homostyly in tetraploid *P. obconica* is controlled by the **S** complex. Thus, this example is comparable with the majority of cases of homostyly in the genus, *P. sinensis* being the exception.

TABLE 7

The constitutions of thrum plants from the cross, long homostyle × thrum, and the ratios expected when these are backcrossed, for the two possible homostyle genotypes

Theory	Constitutions of thrum plants from duplex homostyle × simplex thrum	Ratios expected from backcross		
		Thrums	Homostyles	Pins
A distinct gene-**H**	1 **HHhh Ssss** . . .	6	5	1
	4 **Hhhh Ssss** . . .	2	1	1
	1 **hhhh Ssss**	1	0	1
An allele-S^1	1 SS^1S^1s	1	1	0
	4 SS^1ss	3	2	1
	1 **Ssss**	1	0	1

It will be noted that in the first four families, four pin plants have appeared where none was expected. These were ignored for the purpose of χ^2 calculations. However, they demonstrate the occurrence of double reduction at the locus of the **S** complex. This has an important bearing on the subject of this paper and will be considered later.

5. DISCUSSION

(i) *The structure of the* **S** *complex*

Since homostyly in *P. obconica* is the result of a change within the **S** complex, the above data may be combined with those from similar, but more varied, examples, to contribute to our knowledge of the structure and behaviour of the **S** complex. The data of Ernst (1933 and 1936*a*) on the breakdown of heterostyly in *P. viscosa* and *P. hortensis*, are the most valuable. He found examples of the separation of style from anther characters, giving short and long homostyle plants which were self-fertile. Further abnormal plants showed distinction between pollen grain size and anther height. These were

self-incompatible homostyles. Thus, three groups of characters can be genetically distinguished. These are :

1. Length of style and stigmatic papillæ, and style incompatibility reaction.
2. Anther height.
3. Pollen-grain size and incompatibility reaction.

TABLE 8

Summary of backcross ratios obtained from 19 thrum plants, from the cross, long homostyle × thrum

Thrum parent	Backcross progeny			χ^2	D.f.	P	Ratio tested
	Thrum	Homostyle	Pin				
244^{2}	69	70	2	0·0355	1	0·8-0·9	1 : 1 : 0 Parental thrum $SS^{1}S^{1}s$
245^{4}	55	53	1	0·0459	1	0·8-0·9	
245^{5}	64	59	0	0·2032	1	0·5-0·7	
245^{8}	59	60	1	0·0167	1	0·9	
				0·3013	4		
Total	247	242	4	Deviation 0·0832	1	0·8-0·7	
				Heterogeneity *0·2181*	*3*	*0·98-0·95*	
245^{9}	30	16	11	0·7894	2	0·7	3 : 2 : 1 Parental thrum $SS^{1}ss$
246^{1}	70	39	29	2·6449	2	0·3	
246^{2}	66	62	42	11·3412	2	< 0·01	
246^{3}	69	48	26	0·2867	2	0·9-0·8	
246^{5}	67	45	31	2·5874	2	0·3	
246^{12}	61	41	19	0·0826	2	0·98-0·95	
246^{13}	54	40	25	1·8572	2	0·5-0·3	
246^{18}	80	41	28	2·3221	2	0·5-0·3	
246^{20}	81	55	22	0·8671	2	0·7-0·5	
246^{21}	67	46	20	0·2783	2	0·9-0·8	
246^{23}	75	42	24	0·8297	2	0·7-0·5	
246^{24}	68	44	23	0·0370	2	0·99-0·98	
				23·9236	24		
Total	788	519	300	Deviation 4·6808	2	0·1	
				Heterogeneity *19·2428*	*22*	*0·7-0·5*	
244^{1}	52	0	64	1·2414	1	0·3-0·2	1 : 0 : 1 Parental thrum Ssss
246^{11}	60	0	66	0·2857	1	0·7-0·5	
246^{16}	64	0	69	0·1880	1	0·7-0·5	
				1·7141	3		
Total	176	0	199	Deviation 1·4107	1	0·3-0·2	
				Heterogeneity *0·3034*	*2*	*0·9-0·8*	

Of the eight forms thus possible, Ernst has found all but the self-fertile thrum (see fig. 2), compared with only three forms known in *P. obconica*.

In experiments with the above plants, Ernst showed that the **S** complex is composed of at least three parts, which are tightly linked. These were termed, G, A and P to correspond with the thrum form of the three groups of characters.

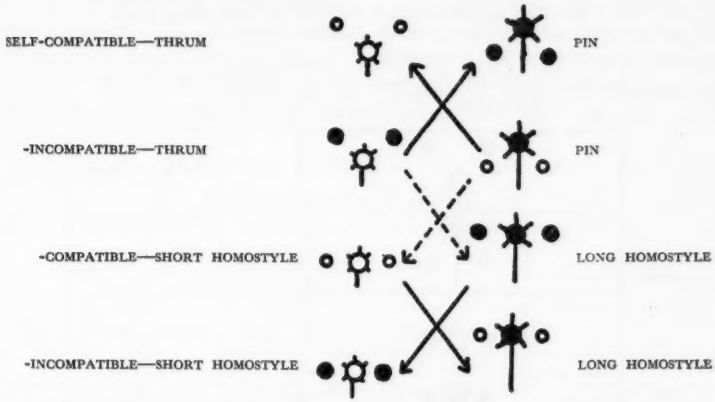

FIG. 2.—The eight possible types of flower structure and their compatibility relationships. These symbols have been modified from the usual ones to show, by difference in size of the ♂ parts, small or large pollen; by appendages on the stigma, short or long papillæ. Shaded parts show a "pin" style reaction or a "thrum" pollen reaction; non-shaded parts show a "thrum" style reaction or a "pin" pollen reaction.
- - - - - - →all anther characters separate from all style characters
————→pollen grain size and incompatibility separate from anther height

Whether the abnormal forms arise by mutation or as a result of crossing-over will be discussed later. If they arise by mutation of parts of the **S** complex, as Ernst believes, then their order is unimportant. If, however, as seems highly probable, they result from crossing-over, then the order in which the units lie on the chromosome

THRUM	PIN
S	**s**
C G S I^s I^p P A	c g s i^s i^p p a
c g s i^s i^p p a	c g s i^s i^p p a

FIG. 3.—The anticipated complete structure of the **S** super gene. C = Thrum area of conducting tissue; G = Thrum style length; S = Thrum style papilla length; I^s = Thrum style incompatibility; I^p = Thrum pollen incompatibility; P = Thrum pollen size; A = Thrum anther height.

is important (see table 9). From the table it seems that the order P—G—A is most unlikely since a self-compatible long homostyle, the commonest abnormality found, requires a double cross-over for its formation. Of G—A—P and G—P—A, the latter is preferable

since only one of its double-crossover types has ever been found. This order has been adopted, therefore, in this discussion.

As pointed out by Lewis (1954) incompatibility is the essence of distyly; the morphology must have arisen subsequently. Hence the S complex must include the original factors, for style and pollen incompatibility reaction. In addition it seems highly probable that

TABLE 9

The three possible orders of sub-genes for eight flower-types.

Flower-type \ Order	1	2	3
	GAP	GPA	PGA
	gap	gpa	pga
	Gap	Gpa	pGa
	gAP	gPA	Pga
	GaP	GPa	PGa
	gAp	gpA	pgA
	(GAp)	(GpA)	(pGA)
	gaP	gPa	Pga

Double cross-over types are boxed in and the type so far undiscovered is in brackets.

factors for length of stigmatic papillæ and for area of conducting tissue also exist. The S gene may, therefore, be conceived as a complex of a number of tightly linked sub-genes (see fig. 3).

(ii) *The mode of breakdown in heterostyly*

Ernst (1936*b*), supposed that abnormal plants had a mutational origin. His scheme includes the suggested origin of all forms, including pins and thrums, from gPA. This is unlikely since breakdown occurs

in established distylic species. The scheme also involves a dominant mutation. However, mutation from GPA must be considered as a possibility. Crossing-over provides an alternative method by which new sub-gene combinations can arise. The fact that seven, out of the eight possible, have been found, and hence the corresponding recombinations, suggests that crossing-over is a likely cause. Observations in *P. obconica* and other heterostyled species support this view.

(a) *Evidence from double reduction.* The appearance of recessives in the progeny of backcrossed triplex plants, which has been noted in *P. obconica*, was first recognised by Blakeslee, Belling and Farnham (1923). Further tetraploid ratios, which differ from expectations on the basis of chromosome segregation, have been recorded since, and

TABLE 10

A survey of examples of double reduction

Author	Tetraploid	Loci	Frequency of double reduction
Blakeslee, Belling and Farnham (1923)	*Datura stramonium*	Purple—white ; P-p Armed—inermis ; A-a	*4·2* per cent. *9·12* per cent.
Lawrence (1929), Lawrence and Scott-Moncrieff (1935)	*Dahlia variabilis*	Yellow—non-yellow ; Y-y	*6·12* per cent.
de Winton and Haldane (1935)	*Primula sinensis*	Green—red ; G-g	*4·8-7·8* per cent.
Mather (1936) . .	*Lycopersicon esculentum*	Red—non-red ; R-r	$\frac{1}{2}(0{\cdot}21 \pm {\cdot}079)$
Fisher (1949) . .	*Lythrum salicaria*	Purple—rosy ; R-r Short—non-short ; S-s	*6·5* per cent. *2·5* per cent.
Fyfe (1953) . .	*Lythrum salicaria*	Mid—non-mid ; M-m	$9{\cdot}878 \pm {\cdot}936$ per cent.

The above values were calculated from triplex backcross data, except for those of Fisher and Mather. They used data from backcrossed simplex plants.

examples of such loci are given in table 10. Darlington (1929) showed that this was due to "*double reduction*, where both chromatids of one chromosome pass into the same nucleus at the second telophase". And, in 1931, he showed that this would result in ratios intermediate between the expectations for random chromatid and diploid segregations. The frequency of double reduction, α, is the "proportion of gametes containing homologous genes from identical meiotic chromatids" (Fisher and Mather, 1943). It is therefore a measure of the distance of the locus from the centromere.

The results in table 8 show that double reduction occurs at the locus of the S complex in tetraploid *P. obconica*. Four recessives appeared among 493 plants from backcrossed triplex thrums ($\mathbf{SS^1S^1s}$).

This is a frequency of 0·81 per cent. recessives, giving an estimate of $\alpha = 3{\cdot}2$ per cent. for ovules. (Double reduction is not evident in normal populations of simplex thrums and pins since it would only be recognised by identifying occasional duplex thrums, thus involving a further generation).

TABLE 11

Breakdown of heterostyly in relation to polyploidy

Species with breakdown of heterostyly					
Polyploid species	No. of abnormal types		Diploid species	No. of abnormal types	
	(*a*) sporadic	(*b*) populations		(*a*) sporadic	(*b*) populations
A. Natural					
P. auricula (2)	7	...	*P. veris* (2)	1	...
P. viscosa (4)	61	...	*P. yunnanensis* (6)	1	...
P. hortensis (4)	1	...	*F. esculentum* (9)	1	...
			L. salicaria (10)	1	...
P. japonica (5)	...	>1	*P. malacoides* (11)	1	...
P. capitellata (6)	...	1	*P. vulgaris* (11)	1	...
P. laurentiana (6)	...	>1			
B. Artificial					
			P. vulgaris (1)	...	2
F. esculentum (7)	3	...	*P. chungensis* (5)	...	>1
L. salicaria (7)	3	...			
P. malacoides (8)	1	...			
P. obconica (12)	2	...			
No. of species with no breakdown			No. of species with no breakdown		
13			43 (3, 5, 6, 12)		
Total *23*	*78*	*>3*	Total *51*	*6*	*>3*
Frequency of abnormal types per species **3·52**					**0·18**

>has been used in the above table where more than one population, but an unknown number, contai abnormal types. The minimum figure has been used in calculations of frequencies. Sources of dat are :—(1) Crosby, 1949*b* ; (2) Darwin, 1869 ; (3) Ernst, 1928 ; (4) 1936*b* ; (5) 1938 ; (6) 1953 ; (7 Esser, 1953 ; (8) Kobel, 1927 ; (9) Schoch-Bodmer, 1934 ; (10) Stout, 1925 ; (11) v. Ubisch, 1923 (12) this paper.

Therefore, crossing-over in the region of the **S** complex does occur in tetraploid *P. obconica*. In *Lythrum*, too, where there is a similar breakdown of heterostyly, double reduction, affecting the **S** and **M** loci is also found (Fisher, 1949 and Fyfe, 1953).

In *P. sinensis*, however, where there is no such breakdown of the **S** complex, de Winton and Haldane (1935) found no double reduction at the **S** locus in 434 plants, indicating that, should it occur, α must

be less than 0·92 per cent. This suggests that centromere interference prevents crossing-over within the **S** locus in this species. It is then unnecessary to assume, as Mather (1950) has done, that the **S** locus lies in an inversion.

(b) *Evidence from polyploidy.* It seems certain that the homostyle in tetraploid *P. obconica* did not arise by the doubling-up of a diploid homostyle. No case of homostyly has been reported in the diploid. In fact, Ernst (1928), examined 8000 plants for homostyles without success, and similarly, I have found none among 5000 plants.

A single tetraploid pin or thrum could not be fertilised, but it is possible that the tetraploid race of *P. obconica* might have arisen from a single doubled-up self-fertile homostyle. In such a case the tetraploids would consist of homostyles and pins only, but thrums are also present. Moreover, the first long homostyle used in this work was a single plant among ten pins and thrums obtained from a nursery. Examination of further tetraploids of the same variety from the same

TABLE 12

Classification of abnormal types resulting from breakdown of distyly. The literature used is cited in table 12

Abnormal types	gPA *	Gpa	GPa	gpA	gPa	GpA
Number found	64	3	6	4	5	0

* The gPA populations are not included.

source showed no other cases of homostyly, so that this could not have been one of a segregating genotype.

Another case of homostyly in tetraploid *P. obconica* was noticed by a nurseryman in the variety Eureka in 1944. A 1940 seed catalogue description shows that it was already a tetraploid variety. All this evidence points to the fact that homostyly arose after polyploidy in *P. obconica*.

Further striking examples of the occurrence of breakdown in heterostyly in artificially polyploid species, are provided by the work of Esser (1953). He remarks upon the occurrence of three homostyles in progeny from his new *Lythrum* tetraploids and three more in progeny of newly formed tetraploid *Fagopyrum*. He states that this may either be the result of spontaneous mutation after colchicine treatment or of a new combination of the sub-genes which may occur as the result of chromosome doubling.

In fact, there is a tendency for breakdown in heterostyly to appear in both natural and artificial polyploid forms. A survey of known examples is given in table 11. The species, *P. hortensis* and *P. viscosa*, in which Ernst found considerable breakdown are naturally polyploid.

It has been shown (Darlington, 1940), that in trivalents, and

therefore presumably in quadrivalents, there is both a change in distribution of chiasmata and an increase in their frequency. Lewis (1954) suggested that the former would increase the chance of crossing over within the S complex. In fact, either or both of these effects could be responsible. There is thus an explanation for the preponderance of breakdown in polyploid heterostyles, assuming a

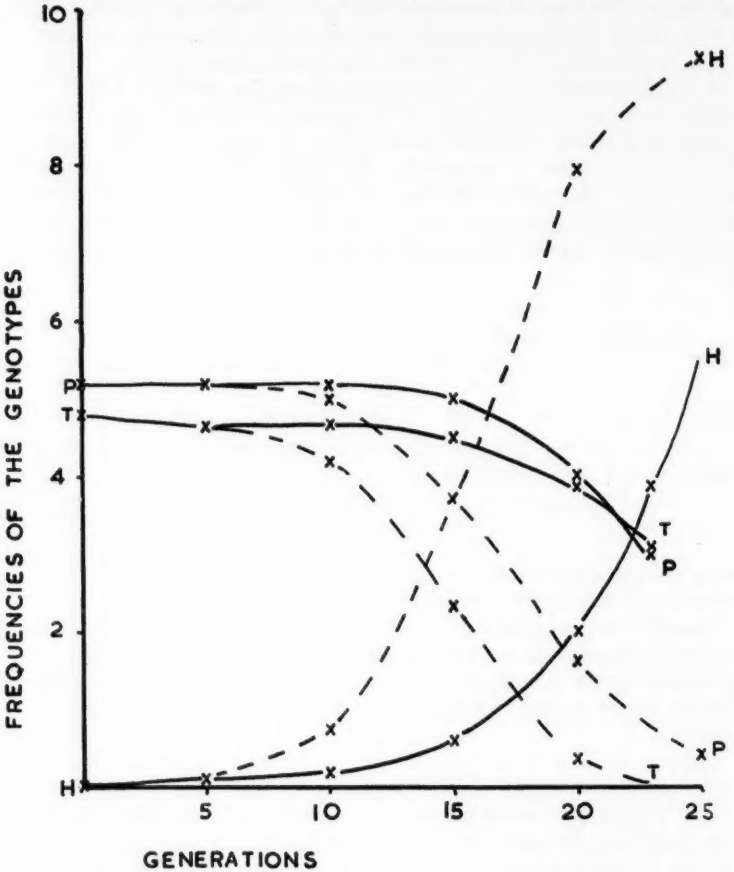

FIG. 4.—Graph comparing the rate of increase of short homostyles with that of long homostyles in similar populations. Generations from arbitrary starting point :—
- - - Crosby (1949) for a population with 1 long homostyle : 499 pins and thrums.
—— for a population with 1 short homostyle : 499 pins and thrums.
T = total thrums P = Pins H = total homostyles

cross-over origin. It is difficult to see how polyploidy could affect mutational breakdown.

(c) *The occurrence of unequal proportions of corresponding cross-over types.* It will be noted that in table 12 there is a high proportion of

the type, gPA, among examples of breakdown in heterostyly. The most striking of these are Crosby's population of *P. vulgaris*, where there are up to 90 per cent. of long homostyles, and Ernst's populations of *P. viscosa*. If these abnormal types are formed by crossing-over it would be expected that gPA and the corresponding cross-over type Gpa, the self-fertile short homostyle, would occur in approximately equal numbers. However, a difference in proportions of the two forms could arise in two ways : (i) if they were of mutational origin, and (ii) if one form had a selective advantage over the other, caused by a difference either in viability or in breeding relationships. The rarity of Gpa in comparison with gPA in thirteen species suggests that it is not simply a question of the unfavourable viability of Gpa.

Among the external forces which will most affect the success of a new genotype are its breeding relationships with members of the population in which it arises. In order to explain the high frequency of gPA, Crosby (1940), devised a theoretical scheme for calculating the approximate trend of a population of 500 plants containing one heterozygous long homostyle, assuming that the relative viability of the homozygotes was 1 (*i.e.* equal to the other types). He found, however, that natural populations of *P. vulgaris* corresponded to a relative viability of 0·65 for the homozygous homostyles.

I have worked out a similar scheme for a population in which one heterozygous short homostyle appears. The same assumptions were adopted, except that the cross, short homostyle × pin, was considered to be unimportant because of the frequent occurrence of selfing in the homostyle. Since thrums are, in this case, involved in crosses with the homostyle, they are of two genotypes, **Ss** and **SS**[1]. Both of these must be considered in each type of cross in which thrums are concerned. This case is thus rather more complex than the *P. vulgaris* one.

The results are summarised in fig. 4. It can be seen that the rate of increase of the short homostyles is much slower than that of long homostyles. They will therefore always be at a disadvantage. Furthermore, if the relative viability of homozygous short homostyles was less than unity, the increase would be even slower, and they might be completely eliminated before ever becoming established.

6. CONCLUSIONS

The morphological characters associated with distyly in *P. obconica* are similar to those in other *Primula* species. In fact, there is evidence that a new one, affecting the fertility of the two forms in *P. obconica*, also occurs in other species. This provides further evidence of the widespread uniformity in distyly.

It is clear that tetraploidy has no effect on the expression of the **S** gene in *P. obconica*. The incompatibility system is maintained in the tetraploid and **S** shows complete dominance in both style and

pollen. Therefore, here, as opposed to gametophytic systems tetraploidy does not result in loss of incompatibility. This is in agreement with the findings in other sporophytic systems (Lewis, 1954). In these there is interaction of alleles in the normal diploid condition, so that polyploidy brings no new situation and no breakdown of the incompatibility system.

The effect of tetraploidy in inducing breakdown of heterostyly, which may result in self-compatibility, is quite distinct from the loss of incompatibility in tetraploids of species with gametophytic control. It involves changes in S gene structure. The fact that the more frequent occurrence of breakdown can be explained by known cytological effects of polyploidy, *i.e.* alterations in number and position of chiasmata, suggests that it is produced by crossing-over. There seems to be no reason to assume an increased mutation rate in polyploids. That crossing-over can occur in this region is shown by double reduction at the S locus in *P. obconica* and at the S and M loci in *Lythrum*.

In *P. obconica*, no genes linked with S have so far been found. It is therefore impossible to prove crossing-over within the S gene. The evidence described supports the view that it does occur. In addition, recent work has shown that a number of multiple-allelic systems are in fact pseudo-alleles or complex genes. From such evidence, the concept that the gene may occupy a chromosome segment larger than the unit of crossing-over is derived. This view makes it even more probable that breakdown of heterostyly is by crossing-over within the S super gene. If this is so, the S complex in *Primula* belongs to the class showing no position effect, since Ernst could make up, for example, thrums of genotype, $\frac{\text{GPA}}{\text{gpA}}$, (a *trans* heterozygote instead of the normal *cis* arrangement). Other examples of this type of complex gene include loci in *Aspergillus*, *Gossypium*, *Maize*, *Oenothera*, *Drosophila*, *Mouse* and *Man* (Pontecorvo, 1952).

The more frequent occurrence of breakdown of the S complex in polyploids suggests a possible breeding technique. If tetraploidy facilitates the breakdown of close linkages by the altered position and frequency of chiasmata in quadrivalents, it could be used as a method of surmounting undesirable tight linkages in breeding work. The possibilities of this method are being investigated with new colchicine-induced tetraploids of *P. obconica*.

7. SUMMARY

1. *Primula obconica* has a distylic breeding system controlled by a single " gene " pair, S—s. In the tetraploid, S is completely dominant.

2. The two mating types are distinguished morphologically by a group of characters. A difference in fertility of the two compatible crosses, pin × thrum and thrum × pin, is shown to be a consequence

of an additional character—a restriction of the area of conducting tissue of pin styles..

3. Breakdown of dimorphic incompatibility has occurred in tetraploid varieties, giving self-fertile homostyles.

4. Homostyly is controlled by the **S** complex. The " allele " is recessive to thrum but dominant over pin (**ss**).

5. This " allele " is probably a new combination of sub-genes of the **S** complex. It could be formed either by intragenic mutation or crossing-over.

6. That crossing-over does occur in this region is shown by double reduction at the locus of the **S** complex.

7. There is evidence that polyploidy induces breakdown of the **S** complex. This can be explained by the change in position and increase of crossing-over in quadrivalents.

8. Corresponding cross-over types are not necessarily expected in equal numbers in the wild, because some have disadvantageous breeding relationships which would eliminate them initially.

9. It is at present impossible to confirm the occurrence of crossing-over within the **S** complex. Evidence favours this as a highly probable cause of breakdown in heterostyly.

Acknowledgments. I wish to express my thanks to Dr D. Lewis, F.R.S. for suggesting this problem and for his continual advice.

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GENETIC RESPONSE TO SELECTION IN CULTIVATED PLANTS: GENE FREQUENCIES IN *PRUNUS AVIUM*

WATKIN WILLIAMS and A. G. BROWN
John Innes Horticultural Institution, Bayfordbury, Hertford, Herts

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I. PREMISES OF THE INVESTIGATION

THE study of allelic frequencies in species of plants with a long history under cultivation affords an excellent opportunity for understanding the interplay of genetic changes which follow evolutionary advance under selection. On the more immediately obvious theoretical grounds, perhaps the most significant single feature to be expected from adaptive advance is a reduction in total genetic variability as represented by the frequency of alternative alleles available at a single locus. In the absence of equilibrating forces, such as heterozygote superiority, or of disturbing linkages with lethal genes which are a constant feature of many types of polymorphism, and excluding cyclic changes in the adaptive values of homozygotes, directional selective trends are expected to achieve genetic fixation of all the loci which affect the adaptive value of the phenotype. In respect of mutant alleles at loci neutral to adaptation, if any exist, their rate of spread would be so slow that little or no variation would ever develop at the locus (Fisher, 1930). Where selection pressure is high, and the state of sexual isolation from divergent populations undergoing adaptation in different directions is almost complete, the amount of allelic variability remaining in populations at equilibrium is expected to be at the extremely low level which only the rate of mutation can sustain.

The ruthless suppression of unfavourable genes and the high degree of reproductive isolation which are the rules of the plant breeder, and the dissection of the population into a series of small, discontinuous, breeding units in the search for maximum phenotypic expression and uniformity, represent a pressure of selection unequalled in nature, except in cases of dominant lethals. It is in cultivated plants and domesticated animals therefore, that one would expect the minimum of allelic variation in respect of all loci irrespective of whether they are adaptive or neutral. This is the thesis that has led Harland on to the notion of genetic erosion during domestication.

A reduction in the available variability is unavoidable under conditions where but a few superior genotypes become the founders of a large specialised population. The importance of the " founder principle " in evolution has been recognised and elaborated notably by Mayr (1942), and its profound effect on improved populations of plants in cultivation is inescapable. Nevertheless the evidence to be presented here shows clearly that the erosion of genetic variability

is not the property of selection at the loci under study. On the contrary the selective advances which have occurred since the domestication of the sweet cherry, appear to have necessitated the conservation of variability through heterozygosis even in what appear superficially as neutral or deleterious genes.

The sweet cherry is an outbreeding diploid with 16 chromosomes and has had a long history under cultivation in Europe and the Near East. Selection under cultivation has probably existed for over 2000 years. In addition, some varieties have had a continuous recorded history for upwards of four centuries. The variety Noble, for example, which is a well-known form in present-day orchards is one which was first described in England in 1611. Under such circumstances only continuous selective trends over very long periods need be considered in relation to frequency levels of allelomorphs at a given locus. Cyclical changes in the environment even if modulated over many years could not be expected to operate in this material. Throughout the cultivated history of the plant selection has been operating for polygenic characters such as season, size and quality of fruit. Fruit colour, which is partly under oligogenic control, has at best probably been subject to direct selective pressure of a very low order. We are therefore concerned here with intensive selection for polygenic complexes seemingly independent of oligogenic variation. The fate of variation at loci with major effects, when selection is for quantitative characters, should prove informative not only in relation to purely plant breeding problems, but also for the understanding of the broader evolutionary issues of polymorphisms and of what Lerner (1953) has termed homeostasis.

Three major gene loci in the European population of cultivated sweet cherry will be considered in this account. They comprise the S locus governing incompatibility, the b locus controlling pigmentation of the sap in the ripe fruit, and the a locus at which albinism is expressed by the homozygous recessive. Information is at present accumulating on a fourth locus where the recessive allele expresses dwarfism, but the data, although pointing towards the same general conclusions are not sufficiently exhaustive to be presented here. All the evidence available indicates that the three loci are independently inherited so that the effects of selection can be studied on sections of three different chromosomes out of the haploid set of eight.

2. FREQUENCIES OF S ALLELES

A total of 125 cherry varieties have been used in tests at the John Innes over the last thirty years, and a total of six incompatibility alleles have been definitely established in nine groups of varieties. Three other definite groups of varieties are known but their precise allelic constitution has not been determined. A thirteenth group has been formed for convenience, containing an assemblage of 20

varieties all of which are compatible with the other 12 groups and most of which are compatible with one another. This is therefore a group of groups, each of the true groups containing but one or, at best, a few varieties. The frequencies of the alleles represented in these latter varieties must necessarily be very low.

The allelic composition of varieties in the nine groups where precise determination of the alleles have been made is as set out in table 1.

Of the 65 varieties represented in the table, 42 or 65 per cent. possess the S3 allele, and the most successful allele in combination with S3 is S1. In this connection it is interesting to note that S1 is itself the second most successful allele of the series, and that S2

TABLE 1

The frequency of the alleles S1-S6 *in 65 varieties of sweet cherry*

	S3	S1	S2	S4	S5	S6	Gene frequency
S3	...	15	10	7	3	7	0·323
S1		...	13	4	0	0	0·246
S2			...	0	2	0	0·192
S4				...	4	0	0·115
S5					...	0	0·069
S6						...	0·054

which also makes a successful combination with S3, is likewise successful when combined with S1. All the remaining combinations, excluding those with S3, appear to have a decided disadvantage. Calculations of expectation in the various genotypic classes are not very helpful in this case, as the estimated gene frequencies have to be calculated from the present population and not from the natural ancestral population from which the material arose. The calculation of Chi is therefore biased in favour of agreement with the observed distribution. The value of χ^2 after correction of values for the obligate absence of homozygotes, gives a value of 4·58 (5 D.F.) which does at least tell us that no major disruptive forces are at work affecting the viability of the genotypes.

That S3 has a strong selective advantage in the cherry can however be supported by a totally different kind of evidence which is not dependent on the initial frequency of the various alleles at this locus. In the course of cherry breeding at the John Innes, Crane and Brown

have selected 9 seedlings which were considered an improvement on existing varieties. It is not irrelevant to this discussion to state here that seven of these cherries are of undoubted merit and have been enthusiastically received by the industry. These seedlings and the genotypic constitution of their parents are set out in table 2.

It is seen from the table that eight out of the nine selected seedlings contain S3. Merton Glory—the only exception—had no S3 in its pedigree. In seven out of the eight selections containing S3 the selector had a choice of either selecting or rejecting S3. The probability of selecting S3 by chance in these eight seedlings is therefore $(\frac{1}{2})^7$ or 0·008. This level of probability is far too low to be ascribed to chance, and offers strong support that S3 itself or the chromosome segment

TABLE 2

The genotypic constitution of selected cherry seedlings and their parents

Seedling	Seedling constitution	Parental constitution
Merton Bigarreau . .	S1·3	S1·2 × S3·4
,, Bounty . .	S1·3	S3·6 × S1·3
,, Favourite . .	S1·3	S1·2 × S1·3
,, Premier . .	S2·3	S3·4 × S1·2
,, Heart . .	S3·6	S1·3 × S3·6
,, Late . . .	S3·4	S1·3 × S3·4
,, Glory . .	?	S1·4 × Sx·y
No. 42	S3·6	S1·3 × S3·6
No. 1464 . . .	S2·3	S2·x × S3·4

carrying S3 has some cryptic effect on economic characters in the cherry and therefore possesses a decided selective advantage.

3. FREQUENCY OF ALLELES AT THE "b" LOCUS

As already indicated the pigmentation of fruit sap in cherries cannot be regarded as entirely free from influences on selection, but under the conditions that have operated on this character, its effect is considered to be very slight. However, this point need not concern us here as the allelic frequency under consideration is not dependent on the operation of selective bias. The pigmentation of sap is governed by two alleles—pigmentation (the so-called black cherries) being dominant to non-pigmentation (white cherries). The recessive genotype can have a varying degree of pigmentation in the skin. The homozygous dominant and heterozygous genotypes are phenotypically identical, and so far genetic tests alone can separate the two pigmented classes. Of the 94 varieties that are or have been in the Bayfordbury collection, 36 per cent. are homozygous recessive. A selection from this collection, at random for all characters except for

their suitability as high quality parents in breeding has given the following analysis :

	Homozygous black (++)	Heterozygous black (+*b*)	Homozygous white (*bb*)
Observed	2	30	19
Expected	7·8	24·2	19

$\chi^2 = 5{\cdot}9$ P 0·02-0·01

If this estimate of the frequency of the two alleles is approximately correct, this sample shows a significant excess of heterozygotes and a deficiency of homozygous dominants. The reported frequency of the recessive phenotype in various collections in different parts of the world have been very variable so that a reliable estimate is difficult to obtain. Further, any estimate within cultivated collections would be of doubtful value, because previous to the present century new varieties originated as chance seedlings from small and widely dispersed plantations when crossing with wild sweet cherries would be more than a possibility. Nevertheless for the 51 varieties reported here, almost all other estimates of the frequency would give a worse fit and the excess of heterozygotes over expected would be increased.

From all the evidence at our disposal there is no indication that there is linkage between the b locus and the incompatibility locus that would explain the deficiency of the homozygous dominant. In all crosses where such linkage might have been detected, *i.e.*

$$\frac{\mathrm{S}x\ \mathrm{B}}{\mathrm{S}y\ b} \text{ or } \frac{\mathrm{S}x\ b}{Sy\ b} \times \frac{\mathrm{S}x\ \mathrm{B}}{Sz\ b}\ \delta$$

no deficiency of one of the classes has been observed. Further the distribution of colour classes with respect of the different S alleles gives no reason to suspect linkage. The similarity between these genotypic frequencies and those of balanced polymorphisms in the Lepidoptera, where one of the homozygotes is kept rare by close linkage with embryonic lethals, forces us to enquire whether such a mechanism might not be operating in the present material. The good agreement with the expected 3 : 1 ratio given by a large number of families in which " b " was segregating precludes this possibility.

We may therefore conclude with reasonable certainty that in the course of selection for quantitative characters in the cherry this seemingly neutral major gene has had a definite selective advantage when in the heterozygous condition. The question of whether one is dealing with the effects of this gene only, or whether it is the result of the co-operative effect of linked genes on a segment on which " b " acts as a marker does not materially alter this simple conclusion.

4. FREQUENCY OF ALLELES AT THE ALBINO LOCUS

Lastly we have to consider the state of variability at the albino locus. The albino factor in this species follows a pattern similar to that described in many species of plants, *i.e.* it is a lethal recessive having all the text-book characters of a major gene. Owing to the long generation time in the cherry only those characters which segregate in the F_1 can be effectively studied in any reasonable period of investigation. Fortunately albinism is one such character, and of 33 varieties which have been used in suitable cross-combinations 17 (51·5 per cent.) are heterozygous for albinism. This analysis shows that the recessive allele occupies 0·26 of the positions available at this locus in this population. The mutant allele being a completely recessive lethal would in the absence of selection achieve equilibrium at a level approximately equal to the square root of the mutation rate. If we are to consider that here we have an allele which is selectively neutral and which has achieved equilibrium, we must also be prepared to believe in a mutation rate from the wild type of the order of 10^{-1}. This becomes clearly an impossible situation, not only from our knowledge of mutation rates, but also from our experience of the stability of the wild type allele in heterozygous varieties which have been under continuous observation over the past three and a half centuries.

In this connection it is interesting to note that Noble, a variety described as originating in this country as far back as 1611 is heterozygous for albinism. Similarly Hedelfingen raised in Germany in 1850, Jaboulay raised in France (1822), Florence raised in Italy (1825), and Semis de Burr raised in the U.S.A. (1844) are all known heterozygotes, indicating that the mutant has had a wide distribution over a long period, and that its presence in high numbers in the present sample is not due to it being exclusively favoured in the English Collection.

In attempting to understand the distribution figures of the two surviving genotypes involving this pair of alleles we may consider two possibilities. Firstly, the present level of the mutant allele may have been conditioned by a process analogous to genetic drift, or more aptly perhaps to Mayr's Founder principle. This means that sometime during its evolutionary history, perhaps during its initial domestication, the population became reduced by selection to a very narrow basis of a few individuals which became the founders of the subsequent population, and that by this process, the allele for albinism achieved accidentally this relatively high frequency. This hypothesis has only one attribute—simplicity, and even if it were valid one would still have to command the assistance of directional selective trends to explain the situation. The minimum population size that could have founded the cultivated cherries is two, and of the four alleles carried at this locus by these parents it is unlikely that the recessive lethal was represented at more than 1. Thus we have at best an initial frequency of 0·25 maintained over 2000 years at a constant level in

spite of the rigorous elimination of the homozygous recessive genotype. Assuming a normal rate of mutation ; merely to maintain a lethal gene at a level of frequency of 0·25 we need a sustained superiority of the heterozygote at a level of about 30 per cent. above the homozygous dominant genotype.

Thus we are forced, irrespective of the functioning of the " founder " principle, to consider the second possibility, namely, the selective advantage of the heterozygote. One has to conclude again therefore that a cryptic selective advantage belongs to the heterozygote of this lethal mutant under the selective pressure which is exercised in the quest for improved expression of quantitative characters of cherries. It is worth noting in relation to this conclusion, that in an intensive study of 75 lethal alleles in *Drosophila melanogaster*, at least three possessed survival advantages in heterozygotes (Stern *et al.*, 1952).

5. DISCUSSION

Before this analysis became possible no one would have suspected the three loci under consideration of having anything but a neutral effect on the desirable fruiting qualities in the cherry. In the case of the incompatibility alleles, the locus is forced by the breeding system into an obligate state of heterozygosis so that discussion here resolves itself around the choice of the effective combination of alleles rather than on the significance of heterozygosis *per se*. Schultz and Briles (1953) have shown the advantage of certain combinations of alleles governing blood groups in poultry where the direction of selection was for increased egg production. These workers also showed that advantageous combinations at one locus were favoured by the epistatic effects of favourable combinations at other loci. Again the discovery of Irwin and his collaborators (1952) of hybrid antigenic substances in some but not in other of their hybrids between species of pigeons and doves, indicates a chemical mechanism through which such combinations could offer advantages. But why should hybrid substances produced at a locus whose only observable expression is confined to the pollen tubes and the styles at reproduction, have any effect on the expression of quantitative characters of importance in a breeding programme? The answer could be pleiotropy or it could be linkage with position effects.

In assessing the likely role of these two possibilities one has to note firstly that the permanent state of heterozygosity and the extent to which allelic variation can develop at this locus afford unique opportunities for the accumulation of mutations on which selection could only act through the heterozygote. Any new mutation in the region close to the S locus would either have to be a dominant mutation (which is unlikely) or would have to possess advantage in the heterozygous condition before it could be used in selection. Therefore heterozygous advantage appears to be a *sine qua non* of linkage of favourable alleles with position effect close to the S locus.

It does not appear necessary on grounds of first principles to invoke linkage when we discover a locus doing something outside the neat role that macrogenetics would ascribe to it for the sake of retaining a " tidy string of beads ". It is inconceivable, specific though gene action may be on the phenotype, that variation at any given locus is without effect on the general biochemical activity of the cell at many stages of developmental processes. One cannot expect a single gene substitution to affect an interdependent series of reactions at one point without affecting the whole series to a greater or lesser extent. In other words new gene substitutions under selection will need altered environments in which to function. These can be provided only by directional responses at other loci to provide the most suitable equilibria for the changed reaction levels. This seems to be the most likely explanation for the allelic frequencies at the S locus in cherries. We are dealing in effect with a correlated response of the genotype following selective changes in other characters—a remote but probably very important form of pleiotropy.

Finally, in respect of the loci for pigmentation and albinism it is difficult to avoid the conclusion that the heterozygotes are superior. In the case of the lethal for albinism it would be exceedingly tortuous to suppose that linked dominant mutants have appeared to enhance the spread of the lethal in the heterozygous condition. Such a development even if it occurred would surely be cancelled by similar mutants on the wild type chromosome before the spread would have proceeded very far. When considering the superiority of the heterozygote it is important to realise that maximal gene effects need not in all cases represent an optimal condition, and heterozygotes with reduced effect may therefore be nearer to the optimum level in any given circumstance. This is clearly seen in the sulphanilamide-requiring strain of *Neurospora* (Emerson, 1948) where the heterokaryon for the suppressor, owing to a reduction in the turn-over of para-amino benzoic acid gives a better growth response. This principle may well be applicable in cultivated plants where gene expression has been pushed to extremes in some directions, and where compensation in other directions is needed to preserve a normal functioning organism. This idea is not new, it has been conveyed in general terms as " integration " (Darlington (1939)), " internal " and " relational " balance (Mather (1943)), " stabilising selection " (Schmalhausen (1949)) and " developmental homeostasis " (Lerner (1954)). Integration in this material appears to be achieved through the heterozygote and not through genetic erosion and fixation. The process proceeds automatically and in parallel with selective advance. This being so, these examples prompt the suggestion that oligogenes play an important polygenic role in the expression of most characters. Indeed may not the basis of quantitative inheritance depend, partly at least, on the interplay of oligogenes ?

6. SUMMARY

1. The frequencies of alleles at the loci which govern incompatibility, pigmentation of the fruit juice, and lethal albinism have been studied in cultivated varieties of the Sweet Cherry—*Prunus avium*.

2. Selection for quantitative economic characters has favoured heterozygotes of the S3 allele at the incompatibility locus, the heterozygote for albinism, and the heterozygote for pigmentation in the fruit juice.

3. These results are discussed in relation to selective properties of apparently neutral, major gene loci, and to the advantages of heterozygosis.

4. The observation is made that the effect of some of the alleles in selection may be due to secondary pleiotropic action on polygenic characters. Pleiotropy in this instance may result from the effects of the alleles modifying the genotype as a whole.

5. The alternative hypothesis of close linkages with groups of polygenes affecting quantitative characters is considered critically, and it is suggested that the present result can be explained without invoking linkage effects.

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INCOMPATIBILITY IN BRACKEN

DAVID WILKIE
Department of Genetics, The University, Glasgow *

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1. INTRODUCTION

In bracken, mature male and female sexual organs are present simultaneously on the same prothallus (fig. 1) so that there is no timing device such as has been found in many other ferns, to prevent self-fertilisation. Preliminary tests suggested that an incompatibility system was in operation and the following is an account of experiments carried out to establish its existence and to elucidate its mechanism.

2. MATERIAL

Three populations of bracken were investigated from the localities of Ballochraggan (population B), Killearn (K) and Milngavie (M) in the south of Scotland. Population M differed morphologically from the other two in that the apices of the pinnæ branched to give rise to the so-called " crested " form ; also the spores were darker in colour. Spore samples were taken from a *single frond* in each case and prothalli were cultured in petri-dishes on agar containing standard Knop solution. Cultures were kept reasonably clean by plating spores which had been washed several times by centrifugation.

3. METHODS

(i) *Preliminary tests*

Populations B and K were investigated separately in the following way : several dishes were plated with spores drawn from a spore sample of the particular population under investigation. Some of the prothalli which developed were isolated individually from one of the dishes into phials before they reached sexual maturity. The prothalli in the remaining dishes were maintained so that when they reached maturity they would give a supply of sperm. Sperm suspensions obtained by flooding these dishes with water are referred to as suspensions of " mixed sperm ". When the isolated prothalli reached maturity they were tested, some against their own sperm and some against mixed sperm. Each prothallus on being flooded once was examined under the dissecting microscope and only those showing the presence of mature archegonia were taken into account, the criterion for maturity being the massing of sperm in the archegonial mucilage (Wilkie, 1954). The results are given in table 1, and this procedure was carried out in all tests.

* Present address : Dept. of Botany, University College, London, W.C. 1.

These results indicate that prothalli have a preference for sperm other than their own. It will be noticed that a certain amount of selfing takes place. This is discussed below.

Prothalli were next isolated in pairs and it was found that in each population approximately 50 per cent. of the pairs were compatible,

TABLE 1

Results of flooding isolated prothalli of two populations with water and with suspensions of mixed sperm

Population	Isolated prothalli			
	Flooded with	No. fertilised	Not fertilised	Total treated
B	Water . . .	8	46	*54*
	Mixed sperm . .	40	8	*48*
K	Water . . .	7	44	*51*
	Mixed sperm . .	41	8	*49*

each member of a pair with the other (table 2). In this experiment only those pairs which had mature antheridia and archegonia in both members at the time of flooding were considered. These results suggest a single-gene, two-allele system of incompatibility in each population.

TABLE 2

Results of flooding isolated pairs of prothalli of each population with water

Population	No. of compatible pairs	No. of incompatible pairs	No. of single fertilisations	Total no. of pairs tested
B	17	8	2	*27*
K	19	12	3	*34*
M	9	9	0	*18*

(ii) *Tests with clones*

For further tests it was necessary to produce clones. This was achieved by sectioning parent prothalli when it was found that regeneration of new prothalli took place from the edges of the segments (fig. 2). These new prothalli were detached, each one into a separate phial and in this way 32 clones were established in all. These were used in series of experiments in which various combinations of clones were tested against each other. The density of sperm suspensions

TABLE 3

Results of testing clones of population M against each other in different combinations

Sperm	Prothalli			
	Clone	Total tested	No. fertilised	Compatibility
Clone 1M	1M	43	3	−
„ 2M		21	1	−
„ 3M		24	2	−
„ 4M		26	1	−
„ 5M		21	0	−
„ 6M		11	9	+
„ 7M		10	0	−
„ 9M		10	1	−
„ 10M		13	11	+
„ 11M		11	1	−
„ 12M		16	15	+
Mixed		*37*	*30*	+
Clone 1M	6M	13	12	+
„ 6M		16	1	−
Mixed		*17*	*15*	+
Clone 1M	7M	13	1	−−
„ 7M		15	0	−
Mixed		*10*	*8*	+
Clone 1M	8M	18	1	−
„ 8M		7	1	−
Mixed		*18*	*15*	+
Clone 1M	9M	9	0	−−
„ 9M		16	1	−
Mixed		*9*	*8*	+
Clone 1M	10M	12	11	++
„ 10M		9	1	−
Mixed		*10*	*9*	+
Clone 1M	11M	20	0	−−
„ 11M		6	0	−
Mixed		*12*	*9*	+
Clone 1M	12M	9	8	++
„ 12M		10	1	−
Mixed		*17*	*14*	+
Clone 1M	13M	10	10	+
„ 13M		8	0	−
Mixed		*9*	*8*	+
Clone 1M	14M	11	1	−
„ 14M		8	0	−
Mixed		*9*	*8*	+
Clone 1M	15M	13	11	+
„ 15M		15	1	−
Mixed		*8*	*7*	+
Clone 9M	9M	11	1	−
„ 11M		18	1	−
„ 12M		17	16	+
Clone 9M	11M	15	0	−−
„ 11M		16	2	−
Clone 6M	6M	13	1	−
„ 12M		17	1	−

−− denotes incompatibility when tested both ways, *i.e.* sexes reversed.
++ denotes compatibility when tested both ways.

used in these experiments was standardised by making hæmocytometer counts followed by appropriate dilution although it was found that sperm densities ranging from 50/ml. to 10^4/ml. gave similar results. The results of these tests are given in tables 3, 4, and 5. Only in those tests in which sperm of clone 6K were used were the results of a doubtful nature. When clone 6K was used as female, on the other hand, there was no doubt as to the results. It appears that the

TABLE 4

Clones of population B tested against each other in different combinations

Sperm	Prothalli			
	Clone	Total tested	No. fertilised	Compatibility
Clone 1B	1B	16	2	−
„ 2B		14	12	+
„ 3B		14	1	−
„ 4B		17	13	+
„ 7B		15	2	−
Clone 1B	2B	27	24	++
„ 2B		21	2	−
Clone 1B	3B	23	2	−
„ 3B		23	3	−
Clone 1B	4B	21	18	++
„ 4B		15	3	−−
Clone 1B	5B	16	14	+
„ 5B		15	1	−
Clone 1B	6B	15	13	+
„ 6B		13	1	−
Clone 1B	7B	20	2	−−
„ 7B		17	3	−
Clone 2B	2B	16	2	−
„ 3B		12	10	+
„ 4B		13	1	−
„ 7B		12	7	+
Mixed		*13*	*9*	+
Clone 3B	3B	13	2	−
„ 7B		22	18	+

mechanism of incompatibility had broken down to a large extent in clone 6K sperm. Otherwise the results listed in the tables indicate the existence of two mating types in each population (the ratios of the two types being 9 : 5 in M, 5 : 4 in K and 3 : 4 in B) the following conditions having been satisfied in each :

(*a*) Two clones both compatible with a test clone are incompatible when tested against each other.

(*b*) Two clones both incompatible with the test clone are incompatible when tested against each other.

(*c*) Two clones, one compatible the other incompatible with the test clone, are compatible when tested against each other. Thus a single-gene, two-allele system may be postulated in each case.

TABLE 5

Clones of population K tested against each other in different combinations

Sperm	Prothalli			
	Clone	Total tested	No. fertilised	Compatibility
Clone 1K	1K	16	2	−
„ 2K		11	10	+
„ 3K		17	15	+
„ 4K		15	1	−
„ 6K		13	1	−
Clone 1K	2K	17	15	++
„ 2K		11	1	−
Mixed		*12*	*10*	+
Clone 1K	3K	18	17	++
„ 3K		12	2	−
Mixed		13	9	+
Clone 1K	4K	19	1	−−
„ 4K		12	2	−
Mixed		*13*	*12*	+
Clone 1K	5K	15	13	+
„ 5K		8	0	−
Clone 1K	6K	13	1	−−
„ 6K		14	4	?
Clone 1K	7K	10	9	+
„ 7K		13	1	−
Clone 1K	8K	17	0	−
„ 8K		14	2	−
Clone 1K	9K	16	13	+
„ 9K		19	2	−
Clone 2K	4K	16	14	+
„ 3K		15	10	+
„ 6K		22	10	?
„ 8K		14	1	−
„ 4K		29	3	−
Mixed		*14*	*12*	+
Clone 4K	6K	15	2	−
„ 6K		19	7	?
Mixed		*10*	*8*	+

When the two mating types of each population were tested against one another they were found to be cross-compatible in all combinations (table 6) so that, if incompatibility is controlled by a single gene there must be multiple alleles. Also there may be more than two

alleles in each population since spore samples were taken from a single frond.

TABLE 6

Results of testing mating types of populations M, B and K against one another in different combinations

Sperm	Prothalli			
	Clone	Total tested	No. fertilised	Compatibility
Clone 3K . . .	3K	22	5	−
„ 4M . . .		23	21	+
„ 6M . . .		25	24	+
Clone 4K . . .	4K	13	2	−
„ 4M . . .		20	19	+
„ 6M . . .		19	17	+
Clone 3B . . .	3B	11	2	−
„ 4M . . .		11	9	+
„ 6M . . .		10	8	+
„ 2K . . .		15	10	+
„ 4K . . .		14	11	+
Clone 4B . . .	4B	12	2	−
„ 4M . . .		14	12	+
„ 6M . . .		10	7	+
„ 2K . . .		10	9	+
„ 4K . . .		10	8	+

4. SELF-FERTILITY

From the relatively high frequency of selfing (table 7) and of crossing between incompatible types, it would appear that the block to self-fertilisation is not a very strong one.

TABLE 7

Comparison of the number of sporophytes produced by selfing with the number obtained from compatible crosses in populations M, B and K

Population	Prothalli flooded with compatible sperm		Prothalli flooded with their own sperm	
	Total	No. fertilised	Total	No. fertilised
B	195	160 (*82* per cent.)	188	23 (*13* per cent.)
K	246	208 (*85* „)	218	33 (*15* „)
M	457	412 (*90* „)	297	22 (*7* „)

Thus the frequency of selfing is of the order of 16 per cent. in B, 17 per cent. in K and 8 per cent. in M.

5. CYTOLOGY

Developing sporangia on the fronds of populations B, M and K were examined cytologically. The chromosomes in meiotic division showed normal pairing and disjunction.

The chromosomes of the gametophyte were studied in developing antheridia, and those of young sporophytes produced in mass culture, in root tips and frond apices. In all three populations a regular alternation of the haploid ($n = 52$) and diploid number of chromosomes took place (fig. 3) indicating a normal sexual cycle.*

The sporophytes produced as a result of selfing in populations B and K were all of normal morphology (fig. 4). Nearly all were examined cytologically and had the diploid number of chromosomes. Self-fertilisation in population M, however, gave rise to sporophytes all of which were weaklings to a greater or lesser degree. Two distinctly abnormal forms occurred and were the subject of special study. One of these forms (fig. 5) was confined to a single clone and selfing in this clone produced only this form of sporophyte. These sporophytes did not develop fronds but produced a cylindrical shoot variously branched. The branches did not have apical meristems but a vascular system was present. The root, on the other hand, was normal and well developed. There was difficulty in keeping these forms alive and the material available for cytological study was limited. The few successful preparations that were obtained showed these to be diploid (fig. 6).

It was concluded that prothalli of this clone carried one or more recessive genes which acted only in the sporophyte and when in the homozygous state resulted in a semi-lethal condition.

The other abnormal form of sporophyte was not confined to a single clone but occurred in several. The clones which produced this form also produced on selfing sporophytes which, though stunted to various degrees, were of normal morphology and had the diploid number of chromosomes. In this second abnormal form, instead of giving rise to fronds as in fig. 4, the apices of the young shoot developed prothallial tissue. Also roots did not develop (fig. 7). Developing antheridia on these prothallial outgrowths and the apices of the young shoots both showed the haploid number of chromosomes (fig. 8). It was concluded that this type of abnormal sporophyte was of apogamous origin.

Apogamy is a permanent feature in the life-history of some ferns and the elimination of nuclear change in these cases is achieved in one of two ways. Apogamy may be followed by apospory as seen for example in the well-known case of *Asplenium Filix-foemina* and in the present investigation, or unreduced spores may be produced as in *Pteris cretica* for example (Manton, 1950). Apogamy has also been

* The most successful squash preparations were obtained by the method of pretreatment with 8-oxyquinoline as outlined by Tjio and Levan, 1950. The material was immersed in a saturated solution of this substance at 18° C. for four hours, then fixed and stained in acetic-orcein.

produced in some ferns by mechanically preventing fertilisation (Lang, 1929 ; Duncan, 1941) but this was not the case here.

6. DISCUSSION

The results obtained from the incompatibility tests in the three populations suggest a single gene, multiple allele system to be in operation similar to that of the bipolar fungi. To be complete the investigation would require the use of genetic markers segregating and recombining with the incompatibility alleles postulated here. It has not been possible yet to obtain marked strains due mainly to the difficulty of raising sporophytes to the stage of sporulation and the length of time this involves.

Due to the relatively high frequency of selfing which takes place, the incompatibility system would appear to be a weak one. As pointed out by Bateman (1952) one would expect to find in nature incompatibility systems continually arising and in all stages of evolution. In systems too weak for the requirements of a species further selective action would be towards a more effective mechanism and hence towards a modified genetic basis. An example of selection for outcrossing (though not for prevention of selfing) is seen in the studies of Pontecorvo (1953) on the genetics of the homothallic ascomycete *Aspergillus nidulans*. The original strains of this fungus were completely self-compatible, mixed inoculi of two strains giving crossed and selfed asci in proportions usually well below the theoretical maximum for random karyogamy (*i.e.* 1 : 1). Mutants were utilised in numerous crosses and new recombinant strains selected. By continuous selection of recombinants, alleles favouring outcrossing (whether present in the original strains or induced by mutation) were automatically selected. Strains of *Aspergillus nidulans* now exist which, though self-fertile, will give 100 per cent. crossed asci when combined with certain other strains. This behaviour, termed by Pontecorvo (1953) " relative heterothallism ", indicates the existence of positive incompatibility, *i.e.* mechanisms of preferential fertilisation not based on the prevention of selfing. It has to be noted that the mechanisms of incompatibility in fungi operate at a level altogether different from those in higher plants, including pteridophytes, as shown here. They operate at the level of karyogamy between nuclei in a common cytoplasm. An exception is seen in cocoa where incompatibility is believed to operate in the embryo sac (see Lewis, 1954).

The condition of partial incompatibility seen in bracken may be adequate in maintaining the necessary interchange of genes and the system may become stabilised at the present evolutionary level. Partial incompatibility of this type may be widespread in ferns where sexual organs develop simultaneously, but to detect it critical tests of the kind described here would have to be carried out.

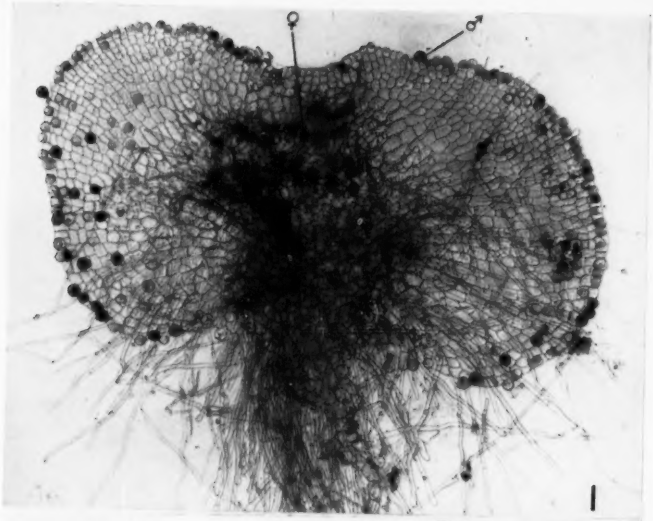
The incompatibility mechanism appears to act after the time of entry of sperm into the archegonial mucilage since no difference in

Plate I

FIG. 1.—Mature prothallus with male (♂) and female (♀) organs. ×30.

FIG. 2.—Regeneration from the edge of a segment of a prothallus. ×20.

FIG. 3.—The diploid (left) and haploid (right) number of chromosomes of the sporophyte and gametophyte respectively. Left ×900 approx., right ×1100 approx.



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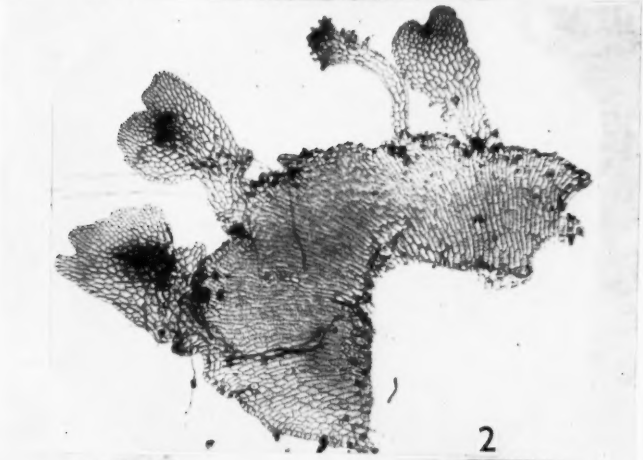

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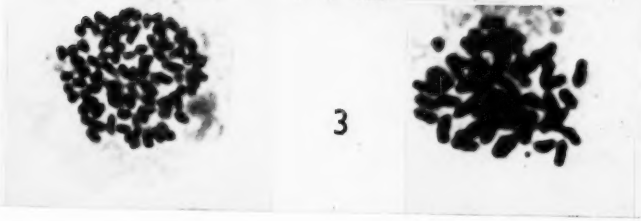

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Plate II

FIG. 4.—A young sporophyte of normal morphology. ×4.

FIG. 5.—Abnormal sporophyte. Shoot (*s*) does not develop fronds but root (*r*) is well developed. ×4.

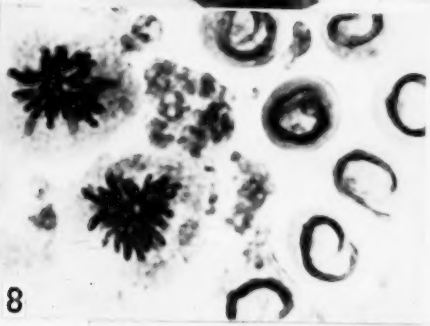
FIG. 6.—Squash preparation of root-tip of 5 showing the diploid number of chromosomes. ×1000 approx.

FIG. 7.—Abnormal sporophyte developing prothallial tissue. ×8.

FIG. 8.—Squash preparation of developing antheridia on 7, showing the haploid number of chromosomes and mature spermatocytes. ×750.

4

5



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6

7

behaviour of compatible and incompatible sperm was detected up to this time. That it acts in the zygote is unlikely since too many sporophytes arise on selfing which are healthy and of normal appearance (at least in populations B and K) for the hypothesis of a lethal condition in the zygote to be tenable. It may be that the barrier arises during the movement of the sperm down through the mucilage in the archegonial neck in analogy to the situation in angiosperms, sperm having to make their way through the mucilage, pollen tubes through the stylar tissue. Lewis (1952) has shown in Oenothera that incompatible pollen tubes carry an antigen the antibody to which is produced in the style so that pollen tube growth is inhibited. It is possible that incompatible sperm carry an antigen the antibody to which is present in the mucilage. A very marked change in the mobility of sperm takes place in the mucilage (Wilkie, 1954) and it is feasible that incompatibility operates at this level.

7. SUMMARY

1. Testing for incompatibility involves the production of clones and the isolation of prothalli.

2. Incompatibility is demonstrated for three populations of bracken, M, B and K.

3. Two mating types have been detected in each of the populations, spores being taken from a single plant in each case.

4. The three populations were cross-compatible in all combinations of mating types and a single gene, multiple allele system of incompatibility is postulated.

5. Incompatibility appears to operate between the time of entry of sperm into the mucilage of mature archegonia and their arrival at the egg surface. Incompatibility acting in the zygote is considered unlikely.

6. Incompatibility alleles appear to be relatively weak in their action since selfing was 8, 16 and 17 per cent. respectively in the M, B and K populations.

7. Each population showed a regular alternation of the haploid ($n = 52$) and diploid number of chromosomes in gametophyte and sporophyte respectively, indicating a normal sexual cycle.

8. Selfing in one of the populations gave rise to two abnormal sporophytes, one diploid, the other haploid.

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CRYPTIC SELF-INCOMPATIBILITY IN THE WALLFLOWER: *CHEIRANTHUS CHEIRI* L.

A. J. BATEMAN

British Empire Cancer Campaign Fellow, Christie Hospital and Holt Radium Institute, Manchester

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I. INTRODUCTION

FROM extensive studies in self-incompatibility in the Cruciferæ (Bateman, 1955) there appeared to be a strong correlation between the breeding system of a species and the conspicuousness of its flowers. These two characters were related in such a way that self-compatible species generally had small and inconspicuous flowers, whereas self-incompatible species achieved conspicuousness. This they did either by the large size of the individual flowers or by their being arranged in conspicuous inflorescences, as in Sweet Alyssum (*Lobularia maritima* L., Desv.).

The correlation is best shown by considering genera containing both breeding systems (table 1). A plausible explanation is that autogamous species have no need to attract insects for their immediate

TABLE 1

Genus	Self-fertile		Self-sterile	
Brassica	*juncea*	O	*campestris*	O
	napus	O	*oleraceus*	O
			nigra	O
Matthiola	*incana*	O	*bicornis*	O
Erysimum	*suffruticosum*	O	*asperum*	O
	cheiranthoides	X	*concinnus*	O
Capsella	*bursa-pastoris*	X	*grandiflora*	O
Alyssum	*alyssoides*	X	*argenteum*	O
	campestre	X	*saxatile*	O
Cardamine	*hirsuta*	X	*pratensis*	O
Arabis	*hirsuta*	X	*albida*	O
Sisymbrium	*officinale*	X	*pinnatifidum*	O
	irio	X		

The correlation between breeding system and flower conspicuousness in genera containing both self-fertile and self-sterile species. O, flowers conspicuous. X, flowers inconspicuous.

survival, but self-incompatible ones are entirely dependent for seed production on some pollinating agent. However, one must not overlook the fact that visually attractive flowers and abundant nectar would in themselves constitute an outbreeding mechanism, albeit a weak one if not reinforced by some barrier to self-fertilisation.*

* The author is not sufficiently familiar with other families to produce a similar table for Rosaceæ, Leguminosæ or Compositæ. However, since these families have diverse additional outbreeding mechanisms based on flower structure, monoecy, apomixis, etc., one would not expect the correlation to be so high.

Among a few striking exceptions to the above rule is the Wallflower, with its self-fertility and very conspicuous flowers; but as I propose to show, in this species at any rate, the exception is only apparent.

FIG. 1.—Arrangement of plants within one open-pollinated plot.
○ = red plant. ● = yellow plant.
Note: the plants are spaced so as to make the plot square.

2. OPEN POLLINATION

Self-pollination of flowers of this species regularly produces a full seed set. Yet casual observation of any bed of Wallflowers in bloom, or even when still only vegetative, discloses a large measure of variation, some of which (such as flower colour), is almost certainly hereditary. This indicates the presence of an outbreeding mechanism of some kind.

The outbreeding was confirmed in experiments in which open-pollinated seed was obtained from single plants of a yellow-flowered variety ("Cloth of Gold") exposed in a bed of red-flowered ones ("Vulcan"). In this material red proved dominant to yellow flowers. Square plots of 100 plants were grown, 10 rows of 10 plants, at three planting densities, the distances between plants being 6 in., 1 ft. and 2 ft. in plots numbered 1, 2 and 3. Planting out was in the autumn. Each plot contained 96 plants of Vulcan and 4 plants of Cloth of Gold, arranged as in fig. 1. It will be seen that each yellow-flowered plant is separated from similar plants by at least four reds and from the outside of the plot by at least two. The proportion of red seedlings in the progeny of such yellow plants will give a good estimate of the degree of natural outcrossing. It will in fact be a minimal estimate, because the following factors will tend to inflate the amount of natural selfing.

Firstly, there will inevitably be a small amount of crossing between yellows (much less than 3 per cent. of the total outcrossing). Secondly, it has been shown (Bateman, 1951) that the behaviour of pollinating insects is such that flower-colour differences between varieties act as isolating mechanisms, so that there would be selective pollination of yellow by yellow. Thirdly, any difference between the varieties in flowering period, or even in the distribution of flowering within the same period, would be an isolating factor.

The three spacings were introduced because the farther apart the individual plants, and the larger they are (size is assumed to be

promoted by wider spacing), the greater will be the ratio of pollinations between flowers on the same plant (geitonogamy) to pollinations between plants.

Owing to late sowing, flowering was not as free as desired, and one of the four yellows in plot 3 did not bloom. At harvesting the fruiting stems of each plant were separated into inner and outer branches. The inner branches should show most geitonogamy. It was found possible to score the progeny at the seedling stage, as the hypocotyls of selfed yellow were acyanic and the hybrids showed purple anthocyanin. Growing-on of a proportion of the seedlings to flowering in the next season confirmed the correctness of the scoring at the seedling stage. One hundred and eight seedlings were scored for each sample, or 216 per plant. The data are presented in table 2.

TABLE 2

Percentage of outcrossing of single yellow Wallflower plants in a plot of red ones

	Plant no.	1	2	3	4	Mean
Plot 1 (6 in. spacing)	Inner branches	80·6	80·6	72·2	68·5	75·5
	Outer branches	89·8	80·6	82·4	51·9	76·2
	Mean	*85·2*	*80·6*	*77·3*	*60·2*	**75·8**
Plot 2 (1 ft. spacing)	Inner branches	76·9	75·0	73·1	not	75·0
	Outer branches	61·1	81·5	75·9	separated	72·8
	Mean	*69·0*	*78·3*	*74·5*	*72·8*	**73·7**
Plot 3 (2 ft. spacing)	Inner branches	64·8	72·2	71·3	no	69·4
	Outer branches	66·7	72·2	64·8	seed	67·9
	Mean	*65·8*	*72·2*	*68·1*	...	**68·7**

Analysis of variance merely confirmed what is apparent on inspection: that position on the plant and planting density are without effect. Although a slight trend in the expected direction appears with increasing spacing, this is only of the same magnitude as the intra-plot variation between plants. The main object of the experiment, however, was to estimate the degree of natural outcrossing. For this the minimal estimate is over 70 per cent. This is remarkably high for a species which, apart from the large flowers and strong scent, seems unadapted for cross-pollination and is self-fertile. Whatever the out-breeding factor may be it is evidently strong enough to swamp completely any effect of geitonogamy.

3. THE DETECTION OF SELF-INCOMPATIBILITY

It occurred to me that in spite of the full seed set on selfing there might be a weak incompatibility reaction which would only show when self pollen had to compete with foreign pollen on its own style, as under open pollination. Such a situation had been anticipated on theoretical grounds (Bateman, 1952). The incompatibility would be detectable only by accurate measurements of pollen tube growth or by mixed pollinations using self and foreign pollen.

The following test was made using only three plants, but its complete explanation of the field data warrants confidence in its validity. Plants numbered 1 and 2 were yellow-flowered, number 3 was red-flowered. Emasculated flowers of plant 1 were pollinated with what we endeavoured to make up as fifty-fifty mixtures of pollen

TABLE 3

Results of mixed pollinations

Pollination type	No. of pollination	No. of flowers pollinated	Progeny		Percentage crossed to red
			Red	Yellow	
1×(2+3)	1	3	33	72	31
	2	6	37	150	20
	3	4	17	74	19
	Total . .	*13*	*87*	*296*	**22·7**
1×(1+3)	4	5	88	11	88
	5	2	56	6	90
	6	5	168	9	95
	7	5	170	15	92
	Total . .	*17*	*482*	*41*	**92·2**

In column "pollination type," 1 and 2 represent different yellow plants; 3 one red plant.

from two plants. The mixtures were of two kinds: (*a*) plants 1 and 3 and (*b*) plants 2 and 3. It is difficult to be sure that any one mixture is properly balanced, but each pollination number in table 3 represents a separate mixture. As earlier, seedlings were classified on hypocotyl colour as red or yellow flowered.

There is a most striking contrast between a deficiency of red progeny when the mixed pollens were both from plants other than the seed parent and the great preponderance of red progeny when the yellow pollen was self pollen. Whatever might be the cause of the deficiency of red in the first series (genetic isolation between varieties?) it makes the excess in the second series all the more striking. This excess is greater than the outcrossing observed in the field. This is probably due to the spatial and temporal advantage of self

over foreign pollen under natural pollination. The magnitude of this advantage would be expected to vary with the activity of the pollinating insects and thus to be very sensitive to the local insect fauna, competing forage crops, and the weather, and also on the number of simultaneously open flowers per plant.

These results have an obvious moral for the plant breeder. It is dangerous to expect self-fertility in the field, merely because in the glasshouse plants give a full seed set on selfing. This danger is likely to be greatest for species which are members of families known to contain much self-incompatibility. Thus cauliflowers are alleged to be self-fertile, but they are suspected of being very prone to contamination by cross-pollination when being grown for seed.

4. SUMMARY

The Wallflower is an apparent exception to the rule in the Cruciferæ that conspicuous flowers are associated with self-sterility. The Wallflower gives a full seed set on being artificially selfed.

However, in the field individual plants show outcrossing of more than 70 per cent.

Experiments with mixed pollinations show that this is because self pollen, though fully capable of fertilisation, cannot compete with foreign pollen, which when present in equal amounts, is responsible for over 90 per cent. of the seed set. There must be a weak self-incompatibility reaction, which under field conditions is still capable of functioning as a moderately efficient outbreeding mechanism.

Note.—The experiments were carried out at the John Innes Horticultural Institution, Hertford.

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LETHAL GENES IN INBRED LINES

J. H. BENNETT
Department of Genetics, University of Cambridge

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1. INTRODUCTION

ISOLATED lethal mutants are of course rapidly eliminated from inbred lines but pairs of completely linked recessive lethal genes lying opposed on homologous chromosomes are able to balance each other and enforce heterozygosity at these as well as at other loci with which they are completely linked. Inbreeding will preserve such balanced lethal systems and the inbred organisms, although alike in genetic constitution, will be maintained in the heterozygous condition at a number of loci.

With lethal mutations not infrequently occurring in nature, it is conceivable that balanced lethal systems might occasionally accumulate and spread through the germ plasm of an inbred line. In the present article, we determine the frequency with which balanced lethal systems can be expected to arise as the result of gene mutation in inbred lines. The rate at which almost-balanced systems of closely linked lethal genes are eliminated from inbred lines is also determined.

2. EXPECTED LIFE OF A RECESSIVE LETHAL GENE IN AN INBRED LINE

A lethal gene can form part of a balanced lethal system in an inbred line only if, during its lifetime in this line, another lethal mutant occurs in an appropriate part of the germ plasm. We shall therefore consider at first the rate of elimination and the expected life of a single lethal gene in an inbred line.

We shall suppose that heterozygotes for lethals and lethal-free homozygotes are equally viable. Only a single mating is required for the continuation of an inbred line and we shall therefore suppose that all matings give rise to the same number of viable offspring.

2.1 *Selfing*

Viable organisms carrying a single recessive lethal gene e at some locus must possess the genotype $l/+$ where $+$ denotes any non-lethal allele of l. With selfing, the probability of occurrence of this genotype is reduced by one-third in each generation. A measure of the rate of elimination of the lethal gene is therefore given by

$$-\log_e(2/3) = 0{\cdot}40546.$$

If u_n is the frequency of the genotype $l/+$ in the n-th generation, the average number of generations required for the elimination of the lethal gene from the line is

$$\frac{\sum_{n=1}^{\infty} n(u_{n-1}-u_n)}{\sum_{n=1}^{\infty} (u_{n-1}-u_n)} = \sum_{n=0}^{\infty} (u_n).$$

Putting $u_o = 1$, we find that a lethal gene will survive under selfing for an average of 3 generations.

2.2 *Sib mating*

Brother-sister matings involving a single lethal gene are of two types, (1) $l/+ \times +/+$ and (2) $l/+ \times l/+$ according as the lethal gene is carried in only one or both of the mated sibs. Suppose these two mating types occur in the n-th generation of sib mating with frequencies u_n and v_n respectively.

The generation matrix is as follows :

	(1)	(2)
$(1)^1$	2	4
$(2)^1$	1	4
column divisor	4	9

From the characteristic equation,

$$(2-4\lambda)(4-9\lambda)-4 = 0$$

we find that the latent roots are

$$\lambda = (17+\sqrt{145})/36 = 0{\cdot}80671$$

and

$$\mu = (17-\sqrt{145})/36 = 0{\cdot}13773.$$

The rate at which sib matings involving a lethal gene are eliminated is thus $-\log_e(0{\cdot}80671) = 0{\cdot}21479$. (With parent-offspring mating, the rate of elimination is $-\log_e(0{\cdot}84576) = 0{\cdot}16572$.) The principal components of frequency (*cf.* Fisher, 1949, p. 30) corresponding with the latent roots λ and μ are respectively,

$$\mathrm{L} = u+(4\lambda-2)v$$
$$\mathrm{M} = u+(4\mu-2)v$$

and since $\mathrm{L}_n = \lambda^n.\mathrm{L}_o$ and $\mathrm{M}_n = \mu^n.\mathrm{M}_o$, it follows that

$$u_n = [(64+84\lambda)\lambda^n.\mathrm{L}_o+(81-18\lambda)\mu^n.\mathrm{M}_o]/145$$
$$v_n = [9(-17+36\lambda)\lambda^n.\mathrm{L}_o-\mu^n.\mathrm{M}_o]/290.$$

The average number of generations for which a mating of type $l/+ \times +/+$ will be present is $\overset{\infty}{\underset{n=0}{S}}(u_n)$. Putting $u_o = 1$ and $v_o = 0$, *i.e.* $L_o = M_o = 1$, we find that the average life of a mating of this type is

$$\frac{1}{145}\left[\frac{64+18\lambda}{1-\lambda} + \frac{18(81-18\lambda)}{1+18\lambda}\right]$$

or $3\frac{1}{3}$ generations. In this case, the mating $l/+ \times l/+$ will be present on the average for $1\frac{1}{2}$ generations, since with $L_o = M_o = 1$,

$$\overset{\infty}{\underset{n=0}{S}}(v_n) = \frac{9}{290}(-17+36\lambda)\left[\frac{1}{1-\lambda} - \frac{18}{1+18\lambda}\right]$$
$$= 1\tfrac{1}{2}.$$

When once present, however, the mating $l/+ \times l/+$ will remain on the average for 3 generations since $\overset{\infty}{\underset{n=0}{S}}(v_n) = 3$ when $u_o = 0$ and $v_o = 1$.

3. FORMATION OF BALANCED LETHAL SYSTEMS

3.1 *Selfing*

We have seen that an isolated recessive lethal gene l survives under selfing for an average of 3 generations. Hence, if at a locus showing no crossing-over with that of the lethal l, lethal mutants l^1 occur at the rate ν per chromosome in each generation, the probability that the genotype l/l^1 will arise following on the appearance of the lethal gene l must be 3ν. All the progeny obtained by selfing individuals of this genotype will also have the genotype l/l^1.

3.2 *Sib mating*

There are four types of brother-sister mating involving both of two lethal genes l, l^1 at two completely linked loci.

(1) $l/l^1 \times +/+$
(2) $l/+ \times l^1/+$
(3) $l/l^1 \times l/+$ and $l/l^1 \times l^1/+$
(4) $l/l^1 \times l/l^1$.

We shall suppose that these matings occur with the frequencies w_n, x_n, y_n and z_n respectively in the n-th generation. The generation matrix is as follows :

	(1)	(2)	(3)	(4)
$(1)^1$	.	2	.	.
$(2)^1$	1	2	2	.
$(3)^1$	.	4	4	.
$(4)^1$	.	1	1	1
column divisor	1	16	9	1

Matings of type (4) are in the balanced condition and can lead only to matings of this same type. There must therefore be a latent root

of unity in the characteristic equation. The principal component of frequency corresponding with this root is

$$Q_n = 3w_n + 6x_n + 10y_n + 38z_n.$$

Since w_n, x_n and y_n each tend to zero with increasing n, the limiting frequency of matings of type (4) is

$$(3w_o + 6x_o + 10y_o + 38z_o)/38.$$

We have seen that after the lethal gene l has appeared, matings $l/+ \times +/+$ and $l/+ \times l/+$ will be present on the average for $3\frac{1}{3}$ and $1\frac{1}{2}$ generations respectively. Now a mutation to l^1 occurring in one of the chromosomes bearing a non-lethal allele of l in the mating $l/+ \times l/+$ will always lead to a mating of type (3). In the mating $l/+ \times +/+$, such a mutation will lead to a mating of type (2) in two-thirds of cases and to a mating of type (1) in one-third of cases. Hence, if at some locus completely linked with that of the lethal l, lethal mutants l^1 arise at the rate ν per chromosome in each generation, the probabilities that matings of types (1), (2) and (3) will arise following on the appearance of the lethal l, are $10\nu/3$, $20\nu/3$ and 3ν respectively.

If the initial mating is type (1), then $w_o = 1$ and the limiting value of z_n as n increases indefinitely is seen to be $3/38$. Initial matings of types (2) and (3) lead to limiting values for z_n of $6/38$ and $10/38$ respectively. Hence, the probability that a sib mating will arrive at the balanced lethal condition at these two loci following on the appearance of the lethal gene l is

$$\nu\left(\frac{10}{3} \times \frac{3}{38} + \frac{20}{3} \times \frac{6}{38} + 3 \times \frac{10}{38}\right)$$
$$= 40\nu/19.$$

In brother-sister matings which are in this condition, certain regions of the germ plasm will be kept heterogeneous and if there is no crossing-over in the affected chromosomes, these regions might be of considerable extent. At loci which are incompletely linked to a balanced lethal configuration, homogeneity will ultimately be attained by continued inbreeding but the approach to this limiting condition will be very slow when the linkage is close (see Bartlett and Haldane, 1937). A special case of balanced lethal genes occurs when these are alleles which yield a viable heterozygote (*cf.* Chesley and Dunn (1936); Hosino (1943)). The presence of balanced lethal *alleles* at a number of closely linked loci would completely stop the approach to homogeneity in that part of the germ plasm since two cross-overs between any pair of successive affected loci would be required before any increase in homogeneity could be achieved.

With natural mutation rates, the probability ($40\nu/19$) that the inbred line will arrive at the balanced lethal condition at two specified loci which are completely linked is so extremely small as to be negligible.

However, if there is no crossing-over in the chromosome concerned, the appropriate value of ν will be the frequency of occurrence of lethal mutants throughout the whole chromosome and this may be as much as 1000 times greater than the natural lethal mutation rate at a single locus. In this case, the probability that the inbred line will arrive at the balanced lethal condition in this chromosome, might be as great as one in a hundred.

4. EXPECTED LIFE OF AN ALMOST-BALANCED LETHAL SYSTEM

The genetical situation exemplified in Muller's original demonstration in 1918 of a " balanced lethal " stock of *Drosophila* with beaded wings, where occasional crossing-over was found to take place between the two lethal genes, would seem to be a more likely result of lethal mutation in inbred lines than the situation we have so far considered. The expected life of a system of incompletely linked lethal genes in the absence of further mutation can be calculated as follows.

4.1 *Selfing*

Let l and l^1 denote lethal genes at two distinct loci with recombination fraction y. Organisms heterozygous for both lethals may be classified into two genotypic classes, (1) $l+/+l^1$ and (2) $ll^1/++$. The generation matrix appropriate to selfing is as follows :

	(1)	(2)
$(1)^1$	$2(1-y)^2$	$2y^2$
$(2)^1$	$2y^2$	$2(1-y)^2$
column divisor	$2+y^2$	$3-2y+y^2$

The characteristic equation is

$$4(1-2y)(1-2y+2y^2)-2\lambda(1-y)^2(5-2y+2y^2)+\lambda^2(2+y^2)(3-2y+y^2)=0$$

and in terms of the recombination fraction y, the dominant latent root takes the following values :

y per cent.	Dominant root λ	$\frac{1}{1-\lambda}$
0	1·00000	∞
0·1	0·99800	500
0·5	0·99001	100·1
1·0	0·98005	50·13
5	0·80628	5·16
10	0·71644	3·53
50	0·44444	1·80

This root is greater than two-thirds only when y is less than 17·894 per cent. and so, when y is greater than this, organisms heterozygous

for both lethals will be eliminated even more rapidly than those with a lethal gene at only one of these loci. However, for two closely linked lethals, *i.e.* for very small values of y, the dominant root is close to unity, being

$$\lambda = 1 - 2y + \tfrac{1}{2}y^2 + O(y^3).$$

Such systems of lethals will therefore be eliminated very slowly and may be said to be almost-balanced. The genotype $l+/+l^1$, when once present, can be expected to survive $1/(1-\lambda)$ generations and when y is small this is approximately $1/(2y)$. With one per cent. crossing-over, for example, the genotype $l+/+l^1$ will survive for an average of fifty generations.

4.2 *Sib mating*

There are eight types of sib mating involving both of two lethal genes l, l^1 at two incompletely linked loci :

(1) $l+/+l^1 \times ++/++$
(2) $ll^1/++ \times ++/++$
(3) $l+/++ \times +l^1/++$
(4) $l+/+l^1 \times l+/++$ and $l+/+l^1 \times +l^1/++$
(5) $ll^1/++ \times l+/++$ and $ll^1/++ \times +l^1/++$
(6) $l+/+l^1 \times l+/+l^1$
(7) $l+/+l^1 \times ll^1/++$
(8) $ll^1/++ \times ll^1/++$.

The generation matrix is shown in table 1. In terms of the recombination fraction y, the dominant latent root takes the following values:

y per cent.	Dominant root λ	$\frac{1}{1-\lambda}$
0	1·00000	∞
0·1	0·99761	418·4
0·5	0·98543	68·63
1	0·97119	34·71
5	0·86947	7·66
10	0·77236	4·39
50	0·65078	2·86

This root is greater than 0·80671, the value of the dominant root obtained in section 2.2 for a single lethal gene and sib mating, only when y is less than about 8 per cent. For very small values of y, the dominant root equals $1-2.4\,y$, approximately, so that in this case a mating of type (6) when once present, will survive for an average of $1/(2.4\,y)$ generations.

With parent-offspring mating, twelve mating types need to be distinguished. It can be shown that when the recombination frequency is greater than about 11 per cent., parent-offspring matings involving both lethal genes are eliminated more rapidly than those with a

lethal gene at only one locus. For two closely linked loci, the dominant latent root is approximately $1\text{-}1{\cdot}6\,y$ so that a mating involving parent and offspring which both have the genotype $l+/+l^1$ will survive, when once present, for an average of $1/(1{\cdot}6\,y)$ generations.

A more complete analysis, taking account of the occurrence of fresh lethal mutants at incompletely linked loci, would be rather complex but it is clear from the above considerations that the probability of an inbred line arriving at the almost-balanced condition at a number of closely linked loci as the result of natural mutation must be so extremely small as to be negligible.

TABLE 1

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
$(1)^1$	.	.	2	$2y(1-y)$	$2y(1-y)$	$4y^2(1-y)^2$	$4y^2(1-y)^2$	$4y^2(1-y)^2$
$(2)^1$	$2y^2$	$2(1-y)^2$	.	$2y^2$	$2(1-y)^2$	$4y^4$	$4y^2(1-y)^2$	$4(1-y)^4$
$(3)^1$	$2(1-y)^2$	$2y^2$	2	$2(1-y)$	$2y$	$8y^2(1-y)^2$	$2(1-2y+2y^2)^2$	$8y^2(1-y)^2$
$(4)^1$	.	.	4	$2(1-y)(2-y)$	$2y(1+y)$	$16y(1-y)^3$	$8y(1-y)(1-2y+2y^2)$	$16y^3(1-y)$
$(5)^1$	$4y(1-y)$	$4y(1-y)$	.	$2y(2+y)$	$2(1-y)^2$	$16y^3(1-y)$	$8y(1-y)(1-2y+2y^2)$	$16y(1-y)^3$
$(6)^1$	.	.	1	$(1-y)^2$	y^2	$4(1-y)^4$	$4y^2(1-y)^2$	$4y^4$
$(7)^1$	.	.	.	$2y(1-y)$	$2y(1-y)$	$8y^2(1-y)^2$	$8y^2(1-y)^2$	$8y^2(1-y)^2$
$(8)^1$	y^2	$(1-y)^2$	.	y^2	$(1-y)^2$	$4y^4$	$4y^2(1-y)^2$	$4(1-y)^4$
column divisor	4	4	16	9	9	$(2+y^2)^2$	$(2+y-y^2)^2$	$(3-2y+y^2)^2$

5. DISCUSSION

Gower, Stadler and Johnson (1946) have reported that of 30 flies taken from a stock of *Drosophila* which had been mated brother-to-sister for 37 generations, 12 contained a recessive lethal gene in the third chromosome and 7 of these had, in addition, a lethal in the second chromosome. The 7 lethals in the second chromosome were said to be identical as were 11 of the 12 lethals in the third chromosome. There is thus no evidence for the existence of balanced lethals in the second chromosome and if balanced lethals were present in the third chromosome, the probability is only 0·006 that as many as 11 out of 12 flies chosen at random would have the one kind of lethal gene. Nevertheless, these authors concluded that in inbreeding there is a strong tendency for lethals to collect and balance one another so retarding further progress towards homozygosis.

The above analysis shows that no such tendency should exist in ordinary inbred stock. However, for any extensive region of the germ plasm where crossing-over cannot occur, there will be a slight but definite tendency for lethals to collect and balance one another in inbreeding. We may therefore conclude that if, in the thirty-seventh generation of these authors' inbred line of *Drosophila*, balanced lethals were in fact present in a region of the germ plasm in which crossing-over in the females was not restricted, the constituent lethal genes were almost certainly present in the original foundation stock

from which the inbred line was derived. Since it would be most unusual for two completely linked lethal genes to be present in the foundation stock when this is chosen at random from a freely interbreeding population, we are forced to the conclusion that Gower *et al.* started their experiment with exceptional material.

In the analysis given above we assumed that the heterozygotes for lethals and the lethal-free homozygotes were equally viable. In *Drosophila* there is some evidence that heterozygotes for lethals are rather less viable than the lethal-free homozygotes (Stern *et al.*, 1952). In this case, the probability of an inbred line arriving at the balanced lethal condition would be even smaller than the value at which we have arrived.

6. SUMMARY

Although isolated lethal genes are eliminated by inbreeding, completely linked lethal genes may be so arranged that they constitute a balanced lethal system which, on inbreeding, is perpetuated. In regions of the germ plasm so affected, inbreeding does not lead to a decrease in the amount of heterozygosity. The frequency with which balanced lethal systems will be established in inbred lines as the result of mutation is calculated using matrix methods. With natural mutation rates, the frequency of occurrence of balanced lethal systems is negligible and it is inferred that even closely inbred material will readily eliminate the lethal mutants. With higher mutation rates due to mutagens, or in regions of the germ plasm where crossing-over cannot occur, the frequency of occurrence of balanced lethal systems might not be negligible. A basis is provided for determining what intensity of mutation would be too great for the lethal mutants occurring in inbred lines to be eliminated.

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COMMENT AND REVIEWS

INBREEDING WHEN HOMOZYGOTES ARE AT A DISADVANTAGE: A REPLY

B. I. HAYMAN and KENNETH MATHER

Agricultural Research Council's Unit of Biometrical Genetics, Department of Genetics, University of Birmingham

In an earlier account (1953) we have considered progress towards homozygosis under inbreeding when homozygotes are at a disadvantage, taking into account a number of mating systems and types of selection. In disagreeing with our analysis Haldane (1956) makes two criticisms: first, that the equations are such as cannot be solved by the matrix methods we used; and second, that our "principal results appear to be incorrect" (p. 56). His paper is confined to a treatment of inbreeding under selfing. So in dealing with the points he raised we too will consider only this one mating system from among the number we discussed in our earlier paper.

Haldane's first criticism must spring from the mistaken belief that because the equations are non-linear they cannot be solved by matrix methods (p. 62). Now the relation between the proportions of genotypes in successive generations is linear apart from a variable factor of proportionality. It is well-known mathematically that the factor of proportionality can be ignored in such a case and the equations solved by matrix methods, provided the factor can be obtained as required from an independent source. Since the frequencies of genotypes in the population must sum to unity we clearly have such an independent source so that matrix methods are fully applicable.

More specifically, Haldane claims (p. 62) that a statement on our p. 168 is equivalent to the false equations

$$p_1 = xp_0 + \tfrac{1}{2}xq_0$$
$$q_1 = \tfrac{1}{2}q_0$$

This claim is of course unwarranted: that these are in fact statements of proportionality is made clear by the footnote and the general formulæ for p_n and q_n given on the same page.

Turning now from our methods to consider our results, we may observe that despite his general charge of incorrectness, Haldane records agreement with us in respect, for example, of so important a result as the critical value of $\frac{1}{2}$ for the relative disadvantage of homozygotes, and nowhere cites a specific example of disagreement. True, on p. 63 he cites us as giving only one equilibrium frequency of heterozygotes whereas he finds that three different equilibria may be reached according to circumstances, which he attributes to insufficient care in our specification of the conditions of selection. In fact a second of his three equilibrium frequencies is explicitly given in our section 5.1 and the remaining one is implicit in the general statements of our section 5.2. As to the specification of conditions of selection his case of "seed selection" is the same as our detailed investigation, our "constant numbers of progeny" is identical with his "selection within lines only" and our "differential survival rates" explicitly includes selection both within and between lines. Indeed by their agreement with

ours, Haldane's rederivation of the equilibrium frequencies bear witness not only to the correctness of our findings, but also to the adequacy of our specification of the conditions of selection.

To demonstrate this point more amply, however, and to bring out at the same time the power of the matrix method, let us complete the investigation suggested in our section 5.2, using the method of our 3.1 and 4.1.

The generation matrix is

	aa	*bb*	*ab*
aa	z	.	$\frac{1}{4}x$
bb	.	w	$\frac{1}{4}y$
ab	.	.	$\frac{1}{2}$

where x, y and 1 are the relative survival rates of *aa*, *bb* and *ab* in segregating families while z and w are the relative survival rates of *aa* and *bb* in true-breeding families. If (p_n, q_n, r_n) are the proportions of *aa*, *bb* and *ab* in the nth generation, this matrix is to be interpreted as $p_{n+1} \propto zp_n + \frac{1}{4}x_n r_n$, etc. reading along the rows. The latent roots and (proportional) frequencies in corresponding equilibrium populations are

$$\begin{array}{ll} z & \mathrm{V} = (1, 0, 0) \\ w & \mathrm{V}' = (0, 1, 0) \\ \frac{1}{2} & \mathrm{V}'' = (s, t, 1) \end{array}$$

where $s = \dfrac{x}{2(1-2z)}$ and $t = \dfrac{y}{2(1-2w)}$ are compound survival parameters for each of the homozygotes.

When $z > \frac{1}{2}$, w the population becomes homozygous *aa*.
When $w > \frac{1}{2}$, z the population becomes homozygous *bb*.
(When $z = w > \frac{1}{2}$ the population becomes mixed homozygous *aa* and *bb*.)
When $\frac{1}{2} > z$, w heterozygosity persists and at equilibrium

$$p = \frac{s}{s+t+1},\ q = \frac{t}{s+t+1} \text{ and } r = \frac{1}{s+t+1}.$$

Haldane's three special cases are

(i) Selection within lines only. $z = w = \frac{1}{4}x + \frac{1}{4}y + \frac{1}{2}$. Then $z = w > \frac{1}{2}$ and heterozygotes disappear. This is the result in Haldane's equation (2) and our 5.1, although in the latter we had the equivalent restriction that $z = w = 1$ and would have divided the third column of the matrix by its sum, $\frac{1}{4}x + \frac{1}{4}y + \frac{1}{2}$.

(ii) Selection between lines only. $x = y = 1$. Haldane's equilibrium results are included in the above inequalities. In particular, if $\frac{1}{2} > z = w$

$$r = \frac{1-2z}{2(1-z)}.$$

(iii) Seed selection. $z = x$ and $w = y$. This is our section 4.1, and Haldane's equations (12) are included. If further $x = y$ (our 2.1) then

$$r = \frac{1-2x}{1-x}.$$

It will be observed that generally the rate of approach to and the kind of equilibrium depend solely on the survival rates, z and w, between lines. When heterozygosity persists, the equilibrium frequencies are determined by s and t so that quite different sets of values of x, y, z and w may lead to the same equilibrium. For instance, when the two homozygotes survive equally, one-third of the equilibrium population is heterozygous for survival rates within and between lines such as $(\frac{1}{2}, \frac{3}{8})$, $(1, \frac{1}{4})$ and $(2, 0)$.

Now since Haldane attributes the correctness of our equilibrium frequencies to certain special conditions we must also show that the population frequencies after n generations of inbreeding can always be obtained from matrices as well as from difference equations.

We can express the initial frequencies (p_0, q_0, r_0) in terms of V, V′ and V″ which then increase at relative rates z^n, w^n and $\frac{1}{2}^n$ as inbreeding progresses. Thus

$$(p_0, q_0, r_0) = (p_0 - sr_0)\mathrm{V} + (q_0 - tr_0)\mathrm{V}' + r_0\mathrm{V}''$$

and after n generations of selfing

$$(p_n, q_n, r_n) \propto z^n(p_0 - sr_0)\mathrm{V} + w^n(q_0 - tr_0) + 2^{-n}r_0\mathrm{V}''$$

or

$$\begin{aligned} p_n &\propto z^n(p_0 - sr_0) + 2^{-n}sr_0 \\ q_n &\propto w^n(q_0 - tr_0) + 2^{-n}tr_0 \\ r_n &\propto 2^{-n}r_0. \end{aligned}$$

Division of each of the quantities on the right-hand side by their sum converts these relations to equalities,

e.g.

$$r_n = \frac{r_0}{(2z)^n(p_0 - sr_0) + (2w)^n(q_0 - tr_0) + (s + t + 1)r_0}.$$

Once again we consider Haldane's special cases.

(i) Selection within lines only. Then $s + t + 1 = 0$ and $r_n = (2z)^{-n}r_0$. This is Haldane's equation (3) with a suitable change of notation.

(ii) Selection between lines only. We obtain Haldane's equations (7) and (10).

(iii) Seed selection. We obtain Haldane's equations (13), (14) and (15). This case, of course, follows directly from the matrix in our 4.1 and it is, therefore, pertinent to expand r_n fully in terms of x and y. Then

$$r_n = \frac{2(1-2x)(1-2y)r_0}{(2x)^n(1-2y)[2(1-2x)p_0 - xr_0] + (2y)^n(1-2x)[2(1-2y)q_0 - yr_0] + (2 - 3x - 3y + 4xy)r_0}.$$

Placing $x = 1 - k$, $y = 1 - l$ and changing the other symbols appropriately to reproduce Haldane's notation gives his equation (15).

We can drive home this point of identity between his and our results even further by supposing the two homozygotes to survive equally. We could then place $x = y$ in the previous form for r_n to produce Haldane's equation (13) but instead let us derive it from the first and very elementary case on our p. 168. Formulæ are given there for differently defined p_n and q_n, q_n being the frequency of heterozygotes. (In the last term of p_n, px has been misprinted for qx.) On dividing the right-hand sides of these

proportionalities by their sum and rearranging we find that the proportion of heterozygotes after n generations is

$$\frac{(1-2x)q}{(1-x)q+(2x)^n[1-2x-(1-x)q]}$$

where q is the initial proportion of heterozygotes. This, with $1-x=k$ is Haldane's equation (13).

It appears to us, therefore, that far from showing us to be wrong, the results of Haldane's heavier treatment have been merely to confirm our earlier findings and in doing so to demonstrate not only the validity but also the mathematical economy of matrix methods in treating the problems of inbreeding under selfing. No doubt the series of papers which he appears to promise will do the same for the other mating systems we discussed in 1953. At the same time, however, we hope that this exposition will have cleared up any obscurities in our original paper and that it has helped towards a clearer understanding of this selection problem and our method of attacking it. Partly for this reason we have taken the opportunity of considering a case more general than those covered by our earlier detailed investigation and by Haldane's but we hope too that in doing so we have provided in terms of s, t, z and w a solution of the population frequencies after n generations of selfing which will be both simple and useful.

Finally, we realise that the relative merits of two mathematical techniques can be of little import to the majority of geneticists who will be interested more in results than in the mathematical investigations behind them. It is important, however, that the geneticist should be able to place confidence in the results and formulæ which are offered for his use, so that a charge of faulty treatment and incorrect results is not lightly to be dismissed. We trust we have amply demonstrated that our findings can command the geneticist's confidence.

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REVIEWS

EXPERIMENTAL DESIGN AND ITS STATISTICAL BASIS. By D. J. Finney. Cambridge University Press. 1952. Pp. xi+169. 68s.

The public is by now perhaps broken in to purchasing an elementary exposition of less than 200 pages for thirty shillings. In time, in fact, we may look back on such a price as cheap ; yet as things stand the attention of publishers should be drawn to the buyers' attitude towards this extraordinary inflation of book prices.

The University of Chicago Press are to be congratulated on production which is quite excellent. From Dr Finney we can be sure of a solid and masterly exposition. In contrast to some of his previous works in this new one the greatest attention has been given to lucidity, and the style is light and easy.

Evidently the author has medical applications largely in view, and it is to be hoped that the book will be widely read by those concerned with clinical trials. The subject of experimental design had, however, a largely agricultural origin, and thinking in academic circles is still so class-conscious that it is with reluctance that the qualified member of a Learned Profession realises that he has anything to learn from a country bumpkin. Finney gives a helpful suggestion when he writes of the technical terms that from its inception have been used in this subject :

> " The words taken over from agricultural research often help the reader to visualize a problem : they must never be thought to limit the application of the methods."

A difficult point of exposition is presented by the factorial principle in experimentation, for in its train come a great wealth both of new theoretical concepts, and of practical " tips and dodges ". Dr Finney has a certain affection for the " fractional replication ", and, rather surprisingly introduces it before his discussion of confounding. For teaching purposes both should, I believe, but especially the first, be introduced to the student with some warnings as to the new element of uncertainty introduced, sometimes to good purpose, and sometimes pointlessly.

This device, like that of the split-plot, is surely an accessory of occasional utility, but as the latter once did is liable to arouse the same sort of attraction as the latest level of the waist-line !

In all ways, however, this is a most attractive little book, and should serve well to spread competent principles of experimentation into new fields.

R. A. FISHER.

POPULATION GENETICS. By C. C. Li. The University of Chicago Press. 1955. Pp. 366. 75s.

This book, which is a revised and somewhat enlarged edition of Professor Li's *Introduction to Population Genetics*, published in 1948 by the National Peking University Press, consists of an exposition of the " statistical study of Mendelian consequences in populations ". The statistical study of quantitative inheritance and experimental work in population genetics

are not included in this survey. Professor Li's account of what is left extends over twenty-three chapters and falls roughly into three parts, the first eight chapters dealing with equilibrium in large random mating populations in the absence of mutation and selection, the next nine chapters with the theory of inbreeding, and the last six chapters with the effects of mutation and selection in finite as well as very large populations.

In the first two chapters the author describes the derivation and application of the " Hardy-Weinberg " law which is simply the binomial distribution of the equilibrium genotypic frequencies for a diallelic locus in a random mating population. Chapter 3 is headed " Genetic Variance and Correlation " and here Professor Li introduces some confusion. On page 29 he describes the genotypic variance as genetic and then partitions this into what he calls an " additive genic " component, which is what Fisher in *The Genetical Theory of Natural Selection* first labelled as genetic variance, and a component due to dominance. However, in other parts of the book where he refers to genetic variance (chapters 8, 11, 21), the author assumes that there is no dominance and so considers genetic variance with its original meaning.

The distinction between genotypic and genetic variance is of fundamental importance in population genetics and the American practice of using several variants of the original terminology paves the way for confusion and error. We shall refer later to the very serious error into which Professor Li is led in chapter 19 as the result of confusing genotypic and genetic variance. After two chapters given to extending the " Hardy-Weinberg " law to sex-linked loci and to autosomal loci with multiple alleles, there is a discussion of its further extension to loci in autopolyploid organisms. It is a great pity that Professor Li here does not give an exact or clear account of inheritance in autopolyploids but writes instead about assumptions of random chromosome and random chromatid segregation, adding at the end that most segregations are " somewhere in between " those expected on these bases. The exact and general treatment of this problem has been available in the genetical literature for several years. The development of the theory of polysomic inheritance owes much to the experimental work with the tetrasomic plant, *Lythrum salicaria*, begun by Fisher and Mather almost twenty years ago, but this work does not involve any assumption about, or evidence for, chromatid segregation being always equational as Professor Li suggests on page 77 of his book.

After dealing with autopolyploids, the author goes on to discuss equilibrium in populations with self-sterility alleles. On page 83 he mentions the obvious fact that the frequency of a self-sterility allele cannot exceed 50 per cent. in any population. On page 350, however, he gives a frequency distribution for self-sterility alleles in which the gene frequency ranges from 0 to 100 per cent. This would seem to be a case where Wright was wrong. In chapter 8 there is a short discussion of the equilibrium situation at a pair of linked loci in a random mating population.

Of the nine chapters devoted to the theory of inbreeding, the first two deal with matrix methods in self-fertilisation and sib mating, the third with equilibrium in populations in which there is some inbreeding, and the remaining six chapters are taken up with the explanation and application of the methods of path coefficients. To the reviewer it seems that far too much space has been given to these correlation methods. However, the

author has followed Sewall Wright's work very closely. At the end of chapter 13, he writes :

> " The novel and simplifying feature of Wright's method, apart from the technique of path coefficients, is the introduction of the gametic variable into the analysis. In physical reality only a zygote can assume a measurable value (such as weight, height, etc.) ; consequently, only the correlation between zygotes can actually be observed. But, by introducing an abstract gametic variable and the abstract correlation between gametes, the analysis becomes much easier."

What Professor Li fails to point out is that the analysis becomes much easier simply because this method cannot, from its nature, take account of the phenomena of dominance and epistasy.

Chapters 18-20 are concerned with the effect of mutation and selection in large populations. On p. 273, where he is considering the increase in the average selective value in a population whose members are experiencing selection, the author makes a very serious error as the result of confusing genotypic with genetic variance. In what he claims is a simplified version of Fisher's fundamental theorem of natural selection, he asserts that the increase in the average selective value of the population per generation is equal to what is, in fact, the genotypic variance of the selective value. This is not said to be limited to random mating populations but that it is erroneous can be seen very readily in the case of a random mating population where, at equilibrium, the genetic variance of the selective value is zero and there is no change in the average selective value, although the genotypic variance will certainly be positive if there is differential genotypic selection. It was, presumably, this same confusion of genotypic and genetic variance which led Dobzhansky recently to ascribe " adaptive value " to selectively balanced polymorphism. On this particular point, Professor Li is apparently not quite convinced for he writes (p. 260) that balanced polymorphism " furnishes plasticity to a species and thus may be of great evolutionary significance ". Plasticity indeed is a very adaptable word and one to which no precise meaning has been given in this context.

The last three chapters of the book are concerned especially with populations of small or finite size. Whilst it is true that in a large random mating population with a fixed environment and constant genotypic selective values, gene frequency will change so that the average selective value for the population will come to be a maximum, the consequence of selection in this particular case should not be erected into a general principle of selection. Selection acts, in fact, on individuals and in some circumstances may lead to a decline in the average selective value of the population. This is not made clear in this book.

In the last few pages of his book, Professor Li writes about Sewall Wright's " adaptive peaks and valleys ". This is a very confused section. On page 347 Professor Li introduces Wright's " $\overline{W}$-surface " and then refers us to Dobzhansky's book, *Genetics and the Origin of Species* for an " excellent non-mathematical presentation of this whole concept ". It should be made clear that Dobzhansky does not, in fact, discuss this " $\overline{W}$-surface " anywhere in his book, although he does refer to the different " W-surface ", whose conception is also due to Wright. Wright's " peaks " and " valleys " have been criticised before but the confusion of genotypic and genic

quantities on which they depend still needs emphasis, and an account such as that given by Professor Li will not help to clarify the position.

The reviewer has concentrated upon some weaknesses of this book because it is important that vague phrases should not be repeated nor further confusion introduced into the literature on population genetics. However, Professor Li's book contains much that is sound and it will certainly repay close study by any serious student of genetics or evolution.

J. H. BENNETT.

GENETICS IN THE ATOMIC AGE. By C. Auerbach. Edinburgh : Oliver and Boyd. 1956. Pp. 106. 8s. 6d. net.

An interesting attempt to popularise genetics, ingeniously illustrated.

WORLD POPULATION AND RESOURCES. P.E.P. (Political and Economic Planning) Report. Published by P.E.P., London. 1955. Pp. 339. 30s.

STANDING ROOM ONLY, THE CHALLENGE OF OVER-POPULATION. By Karl Sax. Boston : Beacon Press. 1955. Pp. 206. $3.

ADAPTIVE HUMAN FERTILITY. By Paul S. Henshaw. London : McGraw-Hill. 1955. Pp. 322. 41s. 6d.

These three books discuss the human population problem. The P.E.P. volume is the well-documented report of a research group. Dr Sax gives us a personal and political as well as scientific judgment based on valuable new evidence. Dr Henshaw is concerned more with education in physiology and birth control.

GENETICAL SOCIETY OF GREAT BRITAIN

ABSTRACTS of Papers read at the HUNDRED AND TWENTIETH MEETING of the Society, held on 9th and 10th APRIL 1956, in the DEPARTMENT OF ZOOLOGY, UNIVERSITY OF EDINBURGH

ACRIFLAVINE RESISTANT MUTANTS OF *ASPERGILLUS NIDULANS*

ETTA KÄFER and J. A. ROPER
Department of Genetics, The University, Glasgow

Three independently obtained mutants, resistant to acriflavine and certain other substances, have been studied. In each mutant resistance is due to mutation in a single gene. Two of the mutant alleles, which confer different degrees of resistance, are semi-dominant. They are located about 23 units distal to the *w* locus and are presumably allelic. A cross involving these two alleles in repulsion gives 0·1 per cent. sensitives. A third mutant allele, unlinked to the first two, is almost completely recessive. This allele confers relatively slight resistance.

Degrees of resistance in haploids and heterozygous and homozygous diploids have been studied. The selection of haploid and homozygous resistant segregants from heterozygotes has provided a useful additional tool in the analysis of mitotic crossing-over and for the location of new markers.

FUNCTIONAL RELATIONSHIPS BETWEEN THREE ADENINELESS MUTANTS IN *ASPERGILLUS NIDULANS*

E. CALEF
Department of Genetics, The University, Glasgow

Three investigated adenineless mutants, out of five located very close to the pabal region, represent three different sites of mutation. These three mutations bear the following functional relationships: two (ad15 and ad17) are partially complementary—*i.e.* in the absence of adenine the diploid repulsion heterozygote shows intermediate growth between the haploid mutant and the wild type. A third (ad13) is allelic to both: the diploids ad13/ad15 and ad13/ad17 do not grow without adenine. As ad13 gives prototrophs in crosses with either of the other two it seems unlikely to be a deficiency or a mutation involving both sites.

MULTIPLE ALLELES AT A LOCUS CONCERNED WITH GLUTAMIC DEHYDROGENASE PRODUCTION IN *NEUROSPORA CRASSA*

J. A. PATEMAN
Department of Genetics, University of Sheffield
and
J. R. S. FINCHAM
Department of Genetics, University College of Leicester

A number of independently induced amination deficient, *am*, mutants are known in *Neurospora*. These strains require certain amino acids for normal growth and lack demonstrable glutamic dehydrogenase activity as a result of mutation at the *am* locus.

Several hundred backmutant wild types have been obtained from the *am* strains S.2929, 32213 and 47305 after irradiation with ultra-violet. There are significant differences in the backmutation rates obtained from different *am* strains.

In the 54 backmutant strains analysed, the mutant locus is within 1 cM of the *am* locus and all 54 strains recovered the ability to produce some glutamic dehydrogenase. Seven of the backmutants possess very low enzyme activity and one has been investigated in detail. This strain grows like a wild type, but possesses 5-10 per cent. normal enzyme activity. No *am* strains were recovered in 1084

ascospores from a low enzyme strain × wild cross and 12 dissected asci show 2 : 2 segregation for high : low enzyme activity. Twenty asci from a low enzyme × *am* cross show a 2 : 2 segregation for low : no enzyme activity. It is considered that low enzyme strain is due to intermediate allele, *am*^{1}, although a closely linked suppressor is not excluded.

EXTRA-NUCLEAR INHERITANCE IN A HOMOTHALLIC FUNGUS

H. SUBAK SHARPE

Department of Genetics, The University, Glasgow

A strain of *Aspergillus glaucus* gives rise to a particular type of clearly distinguishable sector with a certain frequency.

The behaviour of the original and of the sector mycelia, tested in sexual and asexual propagation, discloses a situation not easily explicable in terms of Mendelian inheritance.

Apart from spontaneous sectoring at a low rate, the original type propagates true under all conditions. The rate of change is roughly 5×10^{-11} per micron^{3} of mycelium.

The sector type propagates true when inocula involving large quantities of cytoplasm are used, *i.e.* blocks of mycelium or masses of sexual or asexual spores. But when sexual or asexual spores from the sector type are dilution plated (new colonies arising from single spores) they segregate into sector and original types. The segregations depend on the parent colony's substrate and other factors. Single haploid uninucleate ascospores of sector type generation after generation give rise to sector type colonies whose spores segregate.

A cross-graft technique has been developed using mycelia differing in morphological and biochemical markers. This technique shows that the determinants controlling the sector type reassociate vegetatively with the nuclei formerly present in the original type.

To explain the observations a genetic model based on extra-nuclear selfreplicating particles is proposed.

THE GENETIC CONTROL OF METHIONINE SYNTHESIS IN *SALMONELLA*

S. W. GLOVER

School of Botany, Trinity College, Dublin

Transduction tests involving 32 independently isolated methionine-less mutants of *Salmonella typhimurium* showed that they could be divided into 5 groups. The number of transductions observed in tests between members of the same group is significantly smaller than in experiments involving members of different groups or when phage raised on wild-type bacteria is used.

The results are consistent with the assumption that each transduction group is equivalent to a gene locus and that the different members of a group are " non-identical alleles " and that the small number of transductions between members of the same group represents the infrequent recombination that takes place between pseudoalleles.

Syntrophism tests, growth responses to methionine precursors and chromatographic analysis of culture filtrates show that complete correspondence exists between the grouping based on transduction tests and the grouping by biochemical tests.

The high frequency with which bacteria carrying two auxotrophic markers can be transduced to prototrophy using phage raised on wild-type bacteria indicates that the methionine loci are linked to several loci controlling the synthesis of cysteine and tryptophane.

EXPERIMENTS ON GENETIC ASSIMILATION

K. G. BATEMAN
Institute of Animal Genetics, Edinburgh

It has been suggested by Waddington that selection of an environmentally-induced character produces a genotype which tends to condition the spontaneous appearance of the character. The process has been termed the Genetic Assimilation of an acquired character.

In a number of cases selection of a phenocopy has led to genetic assimilation. Further selection of the assimilated characters without treatment has produced almost true-breeding stocks. Investigation of the genetic basis of these characters indicates that a major gene and penetrance modifiers may be involved in each case, thus suggesting the importance of incompletely penetrating genes in determining the potential direction of assimilation. There is evidence that assimilation has been the result of the lowering of the threshold for expression of a character in the normal environment owing to an increase in the population mean during phenocopy selection.

HOST EFFECT ON THE SIZE OF IMAGOS DEVELOPING FROM TRANSPLANTED OVARIES (*DROSOPHILA MELANOGASTER*)

E. M. PANTELOURIS
Institute of Animal Genetics, Edinburgh

Two inbred lines of *Drosophila melanogaster* produced by selection for size were used in these experiments : line ES_4 of small size and line LZ_5 for large size, the latter carrying the marker *w*. Ovaries from the " large line " were transplanted at the third instar larval stage into " small line " larvae of the same stage. When the operated larvae gave rise to hatching imagos these were mated to males of the " large line." Two groups of offspring were obtained from these matings (scored by the eye marker gene), namely hybrids of the two lines and homozygotes of the LZ_5 line. Measurements (thorax length) of these as well as of control hybrids and LZ_5 were made.

The statistical analysis of the data provided evidence that (*a*) there is some influence of the host on the size of the LZ_5 individuals developing from the transplanted ovaries, making these offspring somewhat smaller than the control LZ_5, but (*b*) the effect is very small.

" RECOVERY " FROM X-RAY MUTATION

K. NORDBACK and C. AUERBACH
Institute of Animal Genetics, Edinburgh

Experiments on the frequencies of sex-linked lethals in first-day and second-day progenies of X-rayed *Drosophila* males suggest that in the testes of irradiated males spermatozoa may " recover " from X-ray induced breaks and point mutations.

THE PRODUCTION OF SEXUAL ISOLATION IN *DROSOPHILA* BY SELECTION

G. R. KNIGHT, ALAN ROBERTSON and C. H. WADDINGTON
Institute of Animal Genetics, Edinburgh

A population consisting of a mixture of ebony and vestigial flies was allowed to mate freely, and was carried on by selecting as parents for the next generation the flies which were either ebony or vestigial, *i.e.* the offspring of matings of like with like. The proportion of cross-matings gradually fell. The significance of such processes for evolutionary theory is discussed.

AN EXPERIMENTAL STUDY OF THE EFFECTS OF INBREEDING AT DIFFERENT RATES ON SIZE IN *DROSOPHILA MELANOGASTER*

E. C. R. REEVE
Institute of Animal Genetics, Edinburgh
and
A. O. TANTAWY
Faculty of Agriculture, University of Alexandria, Egypt

Selection against homozygotes should lead to the retention of more heterozygosity in slowly inbred than in rapidly inbred lines carried to the same theoretical inbreeding level.

To test this, a number of lines of *D. melanogaster* have been inbred at different rates by sib-mating, ½-sib mating, etc., and mean size and viability, and the variance and heritability of size were estimated at stages when all lines had reached roughly the same inbreeding level.

Mean size and viability first began to decline after about 50 per cent. inbreeding, and declined most rapidly after about 70 per cent. The phenotypic variance of size declined most rapidly in the early stages, and did not decrease further after about 60 per cent. inbreeding. The heritability of size declined more slowly than expected on simple theory, in the later stages of inbreeding, and there was a suggestion that less genetic variability was retained in the more rapidly than in the less rapidly inbred lines.

ACCLIMATISATION TO HIGH TEMPERATURES IN INBRED AND OUTBRED *DROSOPHILA SUBOBSCURA*

J. MAYNARD SMITH
Department of Zoology, University College, London

Three inbred lines of *D. subobscura*, and the three types of F_1 hybrid between them were reared at two temperatures, 15° and 25° C., and the time for which adult flies could survive at 33·5° C. in dry air recorded.

Individuals which had been kept at 25° C. either as larvae or as adults survived for much longer than those kept at 15° C. throughout. Two kinds of temperature acclimatisation can occur, a long-lasting "developmental acclimatisation" in individuals kept at 25° C. until emergence and subsequently at 15° C., and a short-lived "physiological acclimatisation" in individuals kept at 25° C. as adults only.

There were no consistent differences between the capacities for physiological acclimatisation of inbred and outbred flies, but outbred flies showed a significantly greater extent of developmental acclimatisation than did inbred ones. Those genotypes which showed the greatest extent of developmental acclimatisation were also the least variable when raised at a given temperature.

These results confirm the hypothesis that organisms which show a greater capacity for regulation during development, as expressed by greater constancy of adult phenotype, are also capable of greater adaptive modification of phenotype in response to changed conditions.

THE ROLE OF MUTATION IN THE MAINTENANCE OF GENETIC VARIATION

G. A. CLAYTON and A. ROBERTSON
Institute of Animal Genetics, Edinburgh

For the first time, evidence is now accumulating from different sources on the rate of production of new quantitative variation, both spontaneous and induced. The units of measurement have to be in terms of variance rather than of rates at individual loci. Such information is of great value in the discussion of the maintenance of variation in natural populations. For abdominal bristles, the spontaneous rate observed is sufficient to be an important factor in this connection. X-rays appear to give a definite increase in the mutation rate but it is as yet too early to discuss in detail the magnitude of the increase.

GENETIC AND DEVELOPMENTAL HOMEOSTASIS

J. M. THODAY
Department of Genetics, The University, Sheffield

Six lines have been run for ten generations from a wild stock of *Drosophila melanogaster*, two selected for high, two for low sternopleural number and two controls. Bilateral asymmetry of sternopleurals was used as a measure of developmental homeostasis and increased with selection, but did not increase in the controls. F_1s between the pairs of selected lines and also between high lines and low lines had asymmetries characteristic of their parents. These results support the view that homeostatic mechanisms deteriorate in selection lines but do not support the view that this is related to increasing homozygosity. It seems more likely that directional selection itself picks out genes for low developmental homeostasis. Other results to be reported agree with this, but they do not support the view that developmental homeostasis as measured is a significant cause of genetic homeostasis.

CYTOLOGICAL ANALYSIS OF TRANSLOCATIONS IN THE MOUSE

B. M. SLIZYNSKI
Institute of Animal Genetics, Edinburgh

Twelve X-ray induced autosomal translocations in the mouse have been studied cytologically. The work was carried out in four steps : (1) cytological confirmation of the presence of a translocation in a semi-sterile strain, (2) analysis of double translocation heterozygotes establishing cytological independence of genetically independent linkage groups, (3) pachytene analysis of single translocation heterozygotes and (4) where two translocations involved the same linkage group, determination by deduction which chromosome carries this group. The following linkage groups have been localised in the following chromosomes : II in 9, III in 14, V in 17, VIII in 18, IX in 16, XI in 15 and XIII in 19. The genetical part of the work has been done by Dr T. C. Carter, Dr M. F. Lyon and Miss R. Phillips, and has been published by them.

LITTER SIZE IN MICE : STUDIES ON SELECTION, INBREEDING, AND CROSSING

D. S. FALCONER
Institute of Animal Genetics, Edinburgh

The response to two-way selection for number born alive in first litters indicates a realised heritability of about 15 per cent. Selection was effective in both directions, but the improvement was very slow ; the mean increased from 7·5 to 8·5 in 14 generations. Inbreeding reduced the mean by 0·5 for each 10 per cent. of inbreeding coefficient. Crosses were made between the best three out of twenty lines at F = 0·84. Litter size in the cross-breds averaged 9·5. The gain by this means was thus double that by selection and was achieved in a shorter time.

THE DEVELOPMENT OF VESTIGIAL-TAIL IN THE MOUSE

HANS GRÜNEBERG
University College, London

Newborn mice homozygous for the recessive gene for vestigial-tail (*vt*) have either no tail at all or, at most, a short stump. The anomaly can be traced back to the 9½ day stage of embryonic development. In the tail buds of vestigial embryos, there is a reduction of the tail gut which is somewhat variable. There is always a massive overdevelopment, both relatively and absolutely, of the neural tube in the

tail which, on its ventral aspect, grows solid excrescences and ultimately divides into 2-4 branches. Normal mouse embryos, between the ages of 9½-11 days, have a hitherto undescribed ventral thickening of the tail ectoderm; this originates from the cloacal membrane, whence it spreads to the tail tip; in 10-days-old embryos the distal part of the thickening has lost contact with its source and resembles in structure the apical ectodermal ridge of the limb buds. That it may have a similar function in regard to tail growth is suggested by the fact that this thickening is greatly reduced in vestigial embryos. The tail is smaller than normal in 10-days-old embryos; a constriction at its base develops in 12-days-old embryos and by the 13th day the final situation is reached.

CYTOGENETICS OF A MOUSE TRANSLOCATION

C. E. FORD, T. C. CARTER and J. L. HAMERTON
Radiobiological Research Unit, Harwell

Male mice heterozygous for Snell's translocation T(5:8)*a* form quadrivalents in only 8 to 30 per cent. of first spermatocytes, all the remainder having 20 bivalents. The quadrivalents are invariably rings with 4 (exceptionally 5) chiasmata at diplotene. There is evidence that the failure of association always occurs in the distal (non-centromeric) arms. The maximum amount of recombination to be expected in these arms is therefore 15 per cent. But Snell reported more than 30 per cent. recombination between *b* and *a* (*a* marks the break); hence *b* must lie in a centromeric arm. He also showed genetically that *b* and *pa* are in opposite arms; hence *pa* also must be in a centromeric arm. The centromeres are very nearly terminal in all mouse chromosomes. It follows that the centromeres must be at the *Sd* end of Group V and at the *b* end of Group VIII.

The disjunctional arrangements at early anaphase lead to the expectation that about 40 per cent., and certainly not more than 50 per cent., of sperm will be viable. Yet new tests confirm Snell's report that fertility of male heterozygotes exceeds 60 per cent. of normal. Possible explanations of the discrepancy will be discussed.

THE POSSIBLE EFFECT OF OUTCROSSING ON THE EVOLUTION OF CHROMOSOME NUMBERS IN ANIMALS

M. FISCHBERG
Department of Zoology and Comparative Anatomy, Oxford

Beatty and Fischberg found 3·4 per cent. heteroploid, mainly triploid, eggs amongst the 3½-day-old embryos in crosses of the inbred lines A, C_{57} and CBA of *Mus musculus*. The percentage of heteroploid eggs from matings within an inbred line was negligible. This difference, which is statistically significant, suggested that heteroploidy is due to outcrossing and could be the first sign of an incompatibility between sperm and egg of individuals with different genetic constitution. If so, one might equally expect heteroploidy to occur in the offspring of crosses between individuals of small isolated populations of one species and to a greater extent amongst the F_1 of species crosses. This phenomenon could in fact link up with the 100 per cent. haploid false hybrids occurring in the F_1 of crosses between certain forms of animals. It is further suggested that, since the first step of this process of increasing incompatibility between sperm and egg occurs in population crosses, it may be a factor in the evolution of chromosome numbers in animals; for here distant crosses are rare. Preliminary crossing experiments with newts of different populations and of different species lend support to this hypothesis.

BLOOD GROUP GENE FREQUENCIES IN IRELAND

G. W. P. DAWSON
School of Botany, Trinity College, Dublin

The gene frequency maps for the ABO blood groups will be explained in terms of the known invasions, settlements and population movements of the last 1000 years. The Anglo-Norman settlements in the east and south and the James I plantations in the north are clearly reflected in these gene frequency maps.

The frequency of Rhesus negative people is higher in the east than in the west of Ireland.

Against this general background an analysis of data of about 10,000 people living in the city and county of Dublin will be discussed. A significantly higher proportion of the people living in the country regions are group A than in the city. The explanation of this may be that the city has received more emigrants from western Ireland, where the frequency of group A is low, than has the surrounding country. The city has been sub-divided into about 200 areas and the data of people living in these areas have been analysed to decide whether there is a pattern of frequencies within the city. An analysis of the data when divided into occupational groups will also be mentioned.

SOME RECENT BLOOD GROUP DISTINCTIONS BETWEEN NEGROES AND WHITES

RUTH SANGER and R. R. RACE
Medical Research Council Blood Group Research Unit, The Lister Institute, London, S.W. 1

I. Within the MNSs and Duffy Systems

Three blood transfusion compatibility problems in which we have recently been involved have disclosed some new racial antigenic differences.

All European blood samples so far tested have the antigen S or s or both. Two allelic genes *S* and *s* provide a perfectly adequate genetic background for the Ss part of the MN system in Europeans. The blood of some negroes (less than 1 per cent.) has neither the antigen S nor the antigen s. We suppose that such people are homozygous for a third allele S^u not found in whites.

All European blood samples so far tested have the antigen Fy^a or Fy^b or both and two allelic genes provided a sufficient genetic background to the Duffy system. Surprisingly, about 70 per cent. of New York negroes have neither antigen. The simplest genetic explanation is that in the negro there is a third allele, *Fy*, not found in whites, with a frequency of about 82 per cent.

II. Within the Rh System

The antigen V is present in the blood of about 40 per cent. of West Africans, 27 per cent. of New York negroes and about 0·5 per cent. of white people : the antigen belongs to the Rh system. The gene *V* can be part of some *cde* and some *cDe* chromosomes ; its precise place in the system is not yet clear.

The blood of 77 per cent. of New York negroes (and over 90 per cent. of West Africans) discloses itself as non-European by being V+ or by being Fy(a-b-).

FUNCTION AND ORGANISATION OF GENETIC MATERIAL

H. KACSER
Institute of Animal Genetics, Edinburgh

Chromosomes are the carriers of certain functions which are revealed by genetic and biochemical analysis. The evidence suggests that these functions are carried out by transfer of surface specificity to large molecules. A model of the organisation of the material composing the chromosomes must account for this transfer as well as for stability and variability. This is possible if the chromosome itself contains specific surfaces. Under special conditions such a system may be duplicated.

AN IMPROVED METHOD OF SELECTIVE BREEDING IN POULTRY

R. OSBORNE

Poultry Research Centre, Edinburgh

In a typical poultry flock a number of sires are each mated to a number of dams and each mating produces several offspring. Individuals may thus be classified by dam families (full-sibs) or sire families (half and full-sibs). It is shown that, theoretically, for improving traits of low heritability, selection of female breeders on the average of the sire family to which they belong may be markedly more efficient than the orthodox methods of selection on individual merit or full-sib average. Such a feature may prove of importance in the large class of poultry populations where trapnesting facilities are limited, since selection for such traits as egg production may be carried out on the basis of pen records with sire families housed as units.

The value of the method declines with increasing heritability but under all circumstances maximum theoretical gains are offered by an index which gives particular weighting factors to each type of family average and individual merit. Even up to heritability values of 50 per cent. maximum weight is to be attached to sire average, less weight to dam average, and least of all to individual performance.